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Consequences of Water Deficit on Metabolism of Legumes

Allan Klynger da Silva Lobato, Gélia Dinah Monteiro Viana,
Gleberson Guillen Piccinin, Milton Hélio Lima da Silva,
Gabriel Mascarenhas Maciel and Douglas José Marques

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Abstract

The aim of this chapter is (i) to define water deficit and its consequences on growth and development of higher plants; (ii) to present the interferences induced on metabolism, including gas exchange, biochemical compounds, and osmotic substances; and (iii) to explain how these alterations will affect the growth, development, and production of leguminous plants. This chapter reports that the performance in leguminous is affected by water deficiency, which can cause lower growth and development. For gas exchange, leaf relative water content, stomatal conductance, and transpiration rate suffered decrease when cultivated under water deficit. Biochemical compounds, such as soluble amino acids, soluble proteins, proline, and abscisic acid (ABA), are intensively modified after a period under water restriction. The results prove that ABA mediates actively and significantly the proline accumulation and consequent osmotic adjustment in *Vigna unguiculata* leaves that were induced to water deficit and rehydration.

Keywords: Abiotic stress, leguminous crops, gas exchange, osmotic adjustment, water deficiency

1. Introduction

The water supplement inadequate in soil is considered one of the limiting factors to the productive potential in several species [1–2]. Water deficit is an abiotic factor that affects the agricultural production with high frequency and intensity, influencing morphological, physiological, and biochemical aspects [3–4].

As in other crops, performance in leguminous is affected by water deficiency, which can cause lower growth and development, with progressive reduction in leaf dry matter [5], moreover, to promote the abortion of flowers during drought periods and to affect the yield significantly [6], with consequent repercussion on production parameters, such as number of grains and pods per plant.

The deficit water is characterized by water losses that exceed the absorption rate and by this way acts directly in the plant–water relations [7–8], depending on intense and exposure period, in addition to promote changes in the cell and molecular pathways [9], whereby accumulation of organic solutes with the carbohydrates and proline [10], differential gene expression of DNA [11], and quantity variation in the photosynthetic pigments, mainly chlorophylls and carotenoids [12], in which the stomata enclosed interfere in photosynthetic rates occur [13].

The osmotic adjustment is considered one of the important mechanisms developed by the plants to tolerate the water deficiency [14], which promotes the protection of the plant cell structures with membranes and chloroplasts [15], as well as avoid the cell toxicity provoked by the free radicals and maximize the water retention in cell inside [16]; besides it has the advantage of using carbohydrates as energy source under severe stress [6].

Drought is directly related to the overproduction of reactive oxygen species (ROS) [17], such as hydrogen peroxide (H_2O_2) and superoxide (O_2^-) [18], which are highly toxic compounds. ROS promote the oxidation of membranes and damage essential organelles such as chloroplasts [19] and mitochondria [20], which result in cell damage or death [21,22].

Ascorbate (ASC) and glutathione (GSH) have essential functions in antioxidant metabolism [23,24] because ASC is used as a substrate [25–27]. In addition, GSH produces ASC and glutathione disulfide (GSSG), which is used to regenerate GSH via glutathione reductase (GR) [28,29].

The soybean is considered a species sensitive to several abiotic stresses [30], when compared with other tropical legumes, such as *Vigna unguiculata* and *Phaseolus vulgaris* [31,32], as well as other species such as *Gossypium hirsutum* and *Sorghum bicolor* [33,34], in which the sensitivity at water deficit can be emphasized, mainly during the growth and development period, which might cause strong reduction in the yield [35]. However, *V. unguiculata* (L.) Walp. is a species tolerant to drought due to rusticity, and it presents large protein content in grain. This crop is frequently found in agricultural areas in Brazil that are under the influence of abiotic stresses. These areas present small rain index and high temperature. In addition, the soil is susceptible to salinity or to fertility loss [36].

2. Objectives

The aim of this chapter is (i) to define what is water deficit and the consequences on growth and development of higher plants; (ii) to present the interferences induced on metabolism, including gas exchange, biochemical compounds, and osmotic substances; and (iii) to explain

how these alterations will affect the growth, development, and production of leguminous plants.

3. Interference of water deficit on growth and development

Lizana et al. [37] while working with two varieties of *P. vulgaris* under water deficit observed paraheliotropic leaf movement, which was previously described by Pastenes et al. [38]. Leaf movements in Arroz and Orfeo subjected to drought were shown and compared. Figure 1B presents the evolution of the movement of leaves after increasing periods of drought, being determined that the variety Arroz is more sensitive than Orfeo [37].

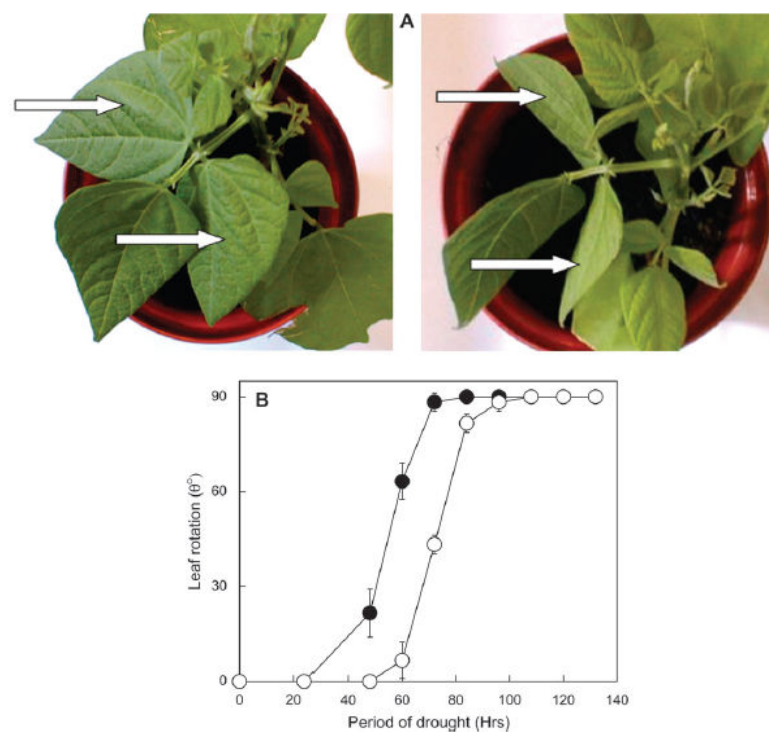


Figure 1. Leaf movement in different drought times. (A) Plants before (left) and after (right) drought-induced leaf movement. Leaf rotation was measured on flanking leaves (arrows) of the first mature trifoliolate leaves. (B) Relationship between period of drought and leaf rotation angle (h) in Arroz (closed symbols) and Orfeo (open symbols) [37].

Lobato et al. [39] while studying morphological alterations in *Glycine max* under progressive water stress found variations in the following parameters: (A) evaluated height of plants, (B) shoot dry matter, (C) number of leaves, and (D) root dry matter.

The lower height and shoot dry matter in the plants under water deficiency occurred, probably due to the abscisic acid (ABA) action, in which case it is produced in the cells under abnormal conditions and this way inhibited the cell division and/or DNA synthesis [39].

The smaller number of leaves showed in the plants under water stress occurred with consequence a lower or void extension rate of the leaf area existent in the plant, moreover probably

increase in the ABA levels in roots, in which it will be transported from roots to shoot and act in the apical region of the plant with antagonist of the auxin and cytokinin, responsible for growth and cell division, respectively [40]; through these hormonal mechanisms, the buds remain dormant and develop not the leaf news. In the period between 0 and 2 days of water stress (Figure 2D), the weight higher of the root dry matter. According to Kerbauy [41], studies with gene-modified plants describe a decrease in the ethylene levels and increase in the ABA in plant roots under water stress, when compared with plants normally irrigated; hence, it proves the different behavior of these hormones, besides it are attributes at ABA the capacity of the remain ethylene normal levels produced in root of plants under normal conditions.

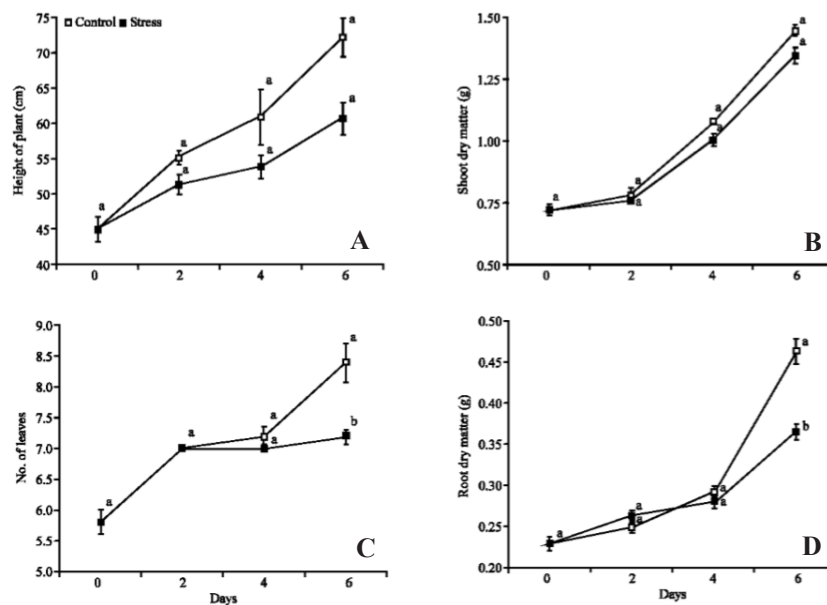


Figure 2. (A) Height of plants, (B) shoot dry matter, (C) number of leaves, and (D) root dry matter in plants of *Glycine max* cultivar sambaiba under 0, 2, 4, and 6 days of water stress. Averages followed by the same letter do not differ among themselves by the Tukey's test at 5% of probability, and the bars represent the mean standard error [39].

4. Modifications on gas exchange

Barbosa et al. [42] evaluated the root contribution to water relations and shoot in two contrasting *V. unguiculata* cultivars and showed that water deficit promoted significant decrease in leaf relative water content (Figure 3A) in tolerant and sensitive cultivars. Inoculated plants of control treatment presented higher values of leaf relative water content, when compared with same treatments of non-inoculated plants.

The tolerant cultivar showed better performance in this parameter, when compared with that of same treatments the cultivar that is sensitive to water stress. In both tolerant and sensitive cultivars, stomatal conductance had a significant reduction in plants exposed to water deficiency (Figure 3B). Plants that were inoculated presented non-significant difference, when compared with that of non-inoculated plants.

Water restriction produced a significant decrease in transpiration rates in both cultivars (Figure 3C). The inoculation provoked non-significant changes in tolerant and sensitive plants. When the tolerant cultivar was submitted to water deficit, the values were higher than those found in the sensitive cultivar, this behavior being similar in inoculated and non-inoculated plants.

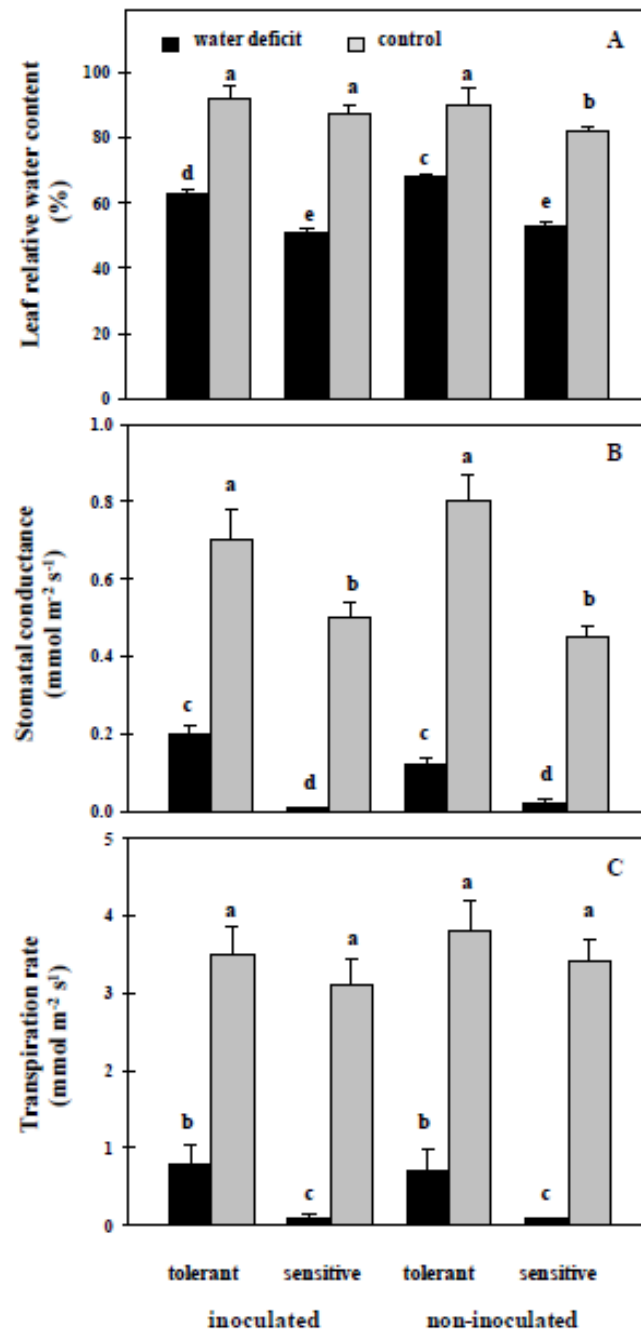


Figure 3. (A) LRWC, (B) g_{sr} and (C) E in two contrasting *Vigna unguiculata* cultivars under water deficit and subjected to inoculation. Means followed by the same letter are not significantly different by the Scott–Knott test at 5% of probability. The bars represent the mean standard error [42].

The reduction in relative water content in leaf is because of lower absorption rate of water by plant via roots and water loss occasioned by gas exchanges through stomata [43]. Similar results were reported by Maia et al. [44] when working with *Zea mays*.

Water deficit promoted a significant fall in stomatal conductance of the two cultivars, but tolerant plants presented higher values of this variable, probably by maintaining better plant water condition. This study revealed that root dry matter exercises influence on stomatal conductance in *V. unguiculata* plants submitted to 5 days of water deficiency, and this fact is based on the indirect effect produced by root on stomatal mechanisms. In other words, an insufficient root system developed during water deficiency will supply lower amount of water to shoot and, consequently, will promote reduction in stomatal conductance.

Decrease in stomatal conductance is explained by reduction in water availability in substrate, and it produces a reduction in leaf water potential, with consequent stomatal closing. The results described by Santos and Carlesso [4] reported that on conditions of water deficit, there is an increase in ABA concentration in xylem sap, promoting stomata closing.

Gholz et al. [45] reported that stomatal closing reduces the CO₂ influx to leaf, affecting production, transport, and utilization of photo-assimilates, and hence the yield. Results similar to those found in this study were found by Santos et al. [46], who studied five *P. vulgaris* genotypes subjected to water deficiency.

Decrease in transpiration rate of *V. unguiculata* plants can be attributed to stomatal behavior, because under water deficit, stomata are kept partially closed, contributing to change in transpiration behavior of plant [47]. Leite and Filho [48] reported that reduction of transpiration is an important mechanism of tolerance to drought. Values of transpiration demonstrated direct relation with stomatal conductance and also with leaf relative water content. Similar results were shown by Nogueira et al. [49] in a study of two *Arachis hypogaea* cultivars exposed to water deficiency.

5. Water deficit on nitrogen compounds

Lobato et al. [50] evaluated the effects of the progressive water deficit, as well as investigated the physiological and biochemical behavior in *G. max* cv. Sambaiba that was submitted to water restriction during the vegetative phase (Table 1). The increase in the levels of free amino acids is due to high synthesis of amino acids from protein hydrolyses, in which case the free amino acids are utilized by the plant to reduce the effects of the water deficit through organic solute accumulation, thereby increasing the water retention capacity [51].

Under water stress, the free amino acids such as proline and glycinebetaine are strongly influenced and consequently quickly accumulated [52], as well as of secondary form occur the increase of aspartate, glutamate and alanine [53]. The result on increase in the free amino acids found by Asha and Rao [54] while working with *Arachis hypogaea* under water deficit corroborates the results of this study.

Free amino acids ($\mu\text{mol g}^{-1}\text{DM}$)		
Days	Control	Stress
0	10.1 \pm 3.5 a	10.1 \pm 3.5 a
2	10.2 \pm 2.8 a	41.6 \pm 3.0 b
4	9.9 \pm 1.9 a	41.2 \pm 3.1 b
6	10.1 \pm 1.5 a	49.3 \pm 2.4 b

Table 1. Free amino acids and proline in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit. Averages followed by the same letter do not differ among themselves by Tukey's test at 5% of probability [50].

The reduction in the total soluble proteins showed in the plants under water stress is due to probable increase in the proteases enzyme activity (Table 2), in which case this proteolytic enzyme promotes the breakdown of the proteins and, consequently, decreases the protein amount presents in the plant under abiotic stress conditions [55]. In inadequate conditions to the plant is active the pathway of proteins breakdown, because the plant use the proteins to the synthesis of nitrogen compounds as amino acids that might auxiliary the plant osmotic adjustment [56]. Similar results on reduction in the proteins were found by Ramos et al. [57], investigating the effects of the water stress in *P. vulgaris*.

Soluble proteins ($\text{mg g}^{-1}\text{DM}$)		
Days	Control	Stress
0	9.74 \pm 0.11 a	9.74 \pm 0.11 a
2	10.05 \pm 0.37 a	7.69 \pm 0.09 b
4	9.87 \pm 0.26 a	7.73 \pm 0.19 b
6	9.71 \pm 0.22 a	7.79 \pm 0.21 b

Table 2. Total soluble proteins in *Glycine max* plants (cv. Sambaiba) under 0, 2, 4, and 6 days of water deficit. Averages followed by the same letter do not differ among themselves by Tukey's test at 5% of probability [50].

6. Relation between abscisic acid and proline

The ABA hormone is synthesized in the plastids and is linked to the stomatal mechanism [58] and quickly responds to water deficiency [59]. The ABA can be produced in the roots and/or shoots, but this hormone is usually synthesized under water deficiency in the roots and translocated to leaves in order to improve stomatal control. The ABA signalization pathway depends on the Ca^{+} influx into the cytosol [60], activating the K^{+} , Cl^{-} , and malate⁻² efflux channels to external medium, through plasmatic membranes and concomitantly blocking the K^{+} entrance to cytosol. Therefore, the cytosol solute flux in the direction of the cell wall results in a decrease of turgescence pressure in the guard cells, and, consequently, the stomata are closed [61].

The progressive increase in ABA concentration in the plants of the stress treatment is related to the stomatal mechanism because this hormone, under these conditions, provokes stomatal closing [62], consequently reducing the water losses during the gas exchanges in essential physiological processes such as transpiration and photosynthesis [59].

Based in study carried out by Costa et al. [5] on impact of water deficit and rehydration on nitrogen compounds and ABA in *V. unguiculata* leaves (Figure 4), the research detected that leaf relative water content influences ABA concentration present in the leaf.

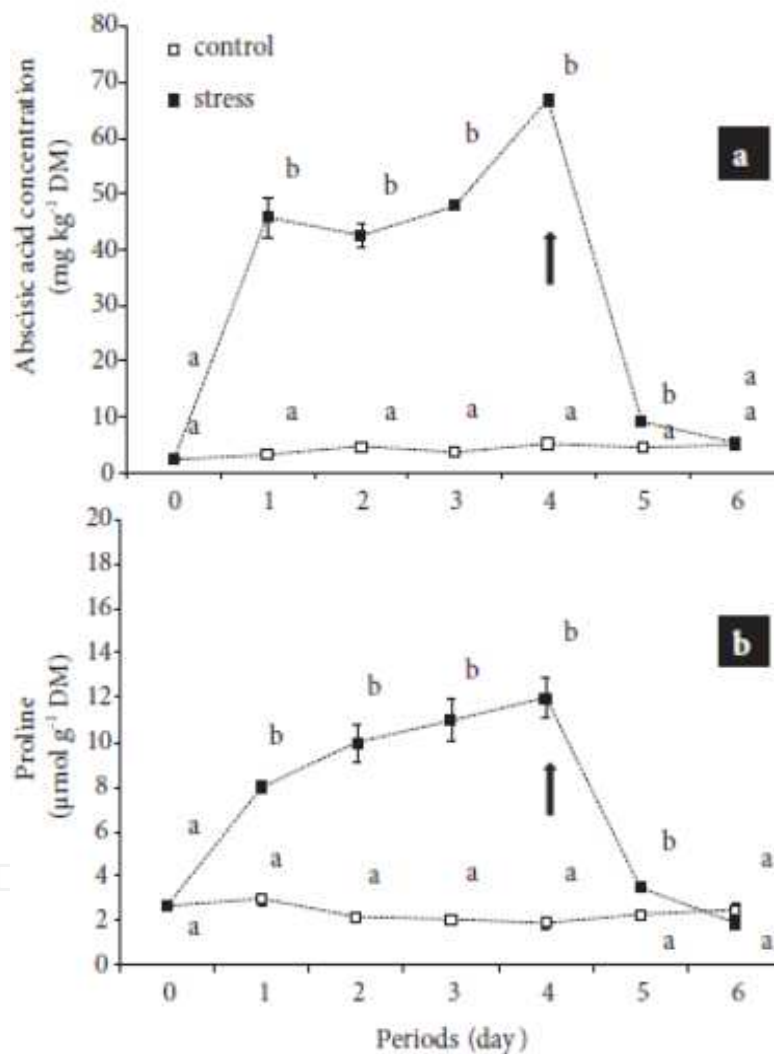


Figure 4. (a) Abscisic acid concentration and (b) proline in *Vigna unguiculata* plants cv. Vita 7 subjected to 4 days of water restriction and 2 days of rehydration. Means followed by the same letter are not significantly different by Tukey's test at 5% of probability. The bars represent the mean standard error, and the arrow indicates the rehydration point [5].

Therefore, the relative water content acts as a signal, and the ABA works during the signal transduction due to the easy and fast movement of this compound into plant tissue, and as a response, the stomatal closing occurs in *V. unguiculata* plants subjected to water deficit.

The fast decrease in the ABA concentration after rehydration indicates the efficiency of the signalization pathway, transduction, and consequent response of this compound. The results reported in this study on ABA are corroborated by Hsu et al. [63] evaluating the consequences of water stress in *Oryza sativa* L. and the effects of heavy metal stress in *Cicer arietinum* L.[64].

The results obtained by Costa et al. [5] prove that ABA mediates actively and significantly the proline accumulation and consequent osmotic adjustment in *V. unguiculata* leaves induced to water deficit and rehydration (Figure 5). A recent study indicated that *V. unguiculata* plants considered resistant to water deficit presented proline accumulation [65] and, consequently, are more adapted to environments with low water supplement, when compared to sensitive plants. The rehydration reduced the proline levels, suggesting that this nitrogen compound participates actively in the osmotic adjustment in this species. The proline accumulation during water deficit presented in this study is similar with results reported by Sarker et al. [66], investigating *Triticum aestivum*, and Smita and Nayyar [67], evaluating *C. arietinum*.

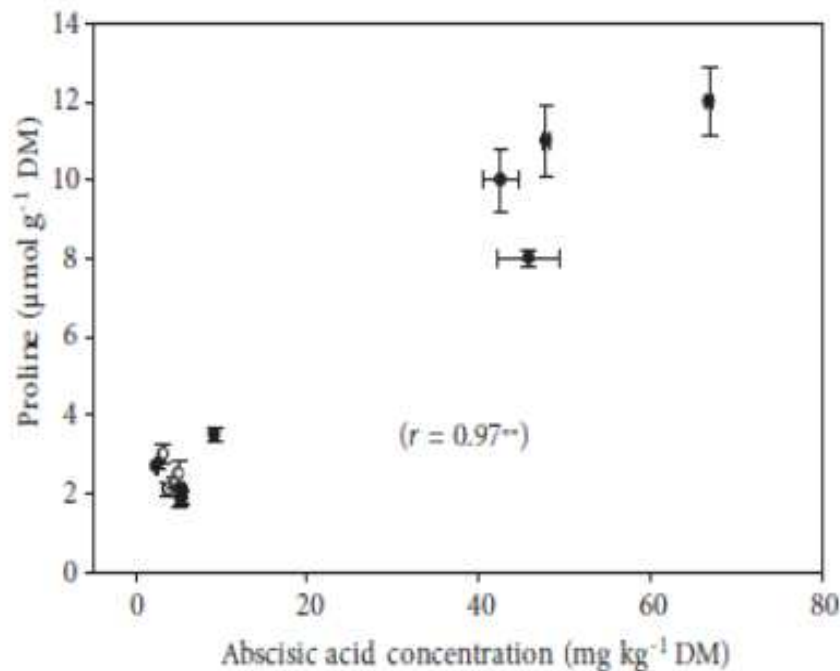


Figure 5. Relationship between abscisic acid concentration and proline in *Vigna unguiculata* plants cv. Vita 7 subjected to 4 days of water restriction and 2 days of rehydration. The bars represent the mean standard error, and the asterisks (**) indicate the significance at 0.01 probability level [5].

7. Final considerations

This chapter reports that the performance in leguminous is affected by water deficiency, which can cause lower growth and development, with progressive reduction in leaf dry matter, moreover to promote abortion of flowers during drought periods, and to affect the yield

significantly, with consequent repercussion on production parameters, such as number of grains and pods per plant. In relation to morphological parameters, negative alterations related to height of plants, shoot dry matter, number of leaves, root dry matter, and paraheliotropic leaf movement were described. For gas exchange, leaf relative water content, stomatal conductance, and transpiration rate suffered decrease when cultivated under water deficit. Biochemical compounds, such as soluble amino acids, soluble proteins, proline, and ABA, are intensively modified after a period under water restriction. The results prove that ABA mediates actively and significantly the proline accumulation and consequent osmotic adjustment in *V. unguiculata* leaves that were induced to water deficit and rehydration.

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Author details

Allan Klynger da Silva Lobato^{1*}, Gélia Dinah Monteiro Viana¹, Gleberon Guillen Piccinin², Milton Hélio Lima da Silva³, Gabriel Mascarenhas Maciel⁴ and Douglas José Marques⁵

*Address all correspondence to: allanllobato@yahoo.com.br

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

2 Campus de Parauapebas, Universidade Federal Rural da Amazônia, Belém, Brazil

3 Coordenação de Botânica, Museu Paraense Emílio Goeldi, Belém, Brazil

4 Instituto de Ciências Agrárias, Universidade Federal de Uberlândia, Monte Carmelo, Brazil

5 Setor de Olericultura e Experimentação, Universidade José do Rosário Vellano (UNIFENAS), Alfenas, Brazil

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