The aim of this study was to evaluate the tolerance of Gabiroba plants (Campomanesia spp) to drought and flooding stresses for 0, 3, 6, 9, 12, 15, and 18 days. The contents of dry matter, water, nitrate reductase activity, total soluble sugars, sucrose, starch, insoluble and soluble protein, and soluble amino acid in leaves and roots were periodically determined. Drought and flooding induced a significant reduction in the contents of water and dry matter of leaves and roots. The contents of total soluble sugars, sucrose, and starch suggest a reduction in the photosynthetic rate, remobilization of starch reserves, and transport blocking in the phloem. Drought stress leads to decreased protein synthesis and accumulation of amino acids. The higher concentration of insoluble proteins in the leaves and roots of plants under flooding is a consequence of the synthesis of new polypeptides. For the leaves, the lowest and highest contents of amino acids were found under drought and flooding stresses, respectively; whereas for the roots, the lowest rates were found under drought stress. Gabiroba plants are tolerant to drought stress and remobilize soluble carbohydrates from the shoots to the roots.

ORIGINAL ARTICLE

Impact of drought and flooding stresses on Gabiroba plants

Emiliane dos Santos Belo1*
Antônio Paulino da Costa Netto1
Vanessa Cristina Stein1
Samuel Mariano Gisol da Silva1

1Universidade Federal de Goiás – UFG, Campus de Jataí, Jataí, GO, Brasil
*Corresponding Author:
E-mail: emiliane.belo@gmail.com

KEYWORDS
Water
Abiotic stress
Oxygen
Tolerance

ABSTRACT: The aim of this study was to evaluate the tolerance of Gabiroba plants (Campomanesia spp) to drought and flooding stresses for 0, 3, 6, 9, 12, 15, and 18 days. The contents of dry matter, water, nitrate reductase activity, total soluble sugars, sucrose, starch, insoluble and soluble protein, and soluble amino acid in leaves and roots were periodically determined. Drought and flooding induced a significant reduction in the contents of water and dry matter of leaves and roots. The contents of total soluble sugars, sucrose, and starch suggest a reduction in the photosynthetic rate, remobilization of starch reserves, and transport blocking in the phloem. Drought stress leads to decreased protein synthesis and accumulation of amino acids. The higher concentration of insoluble proteins in the leaves and roots of plants under flooding is a consequence of the synthesis of new polypeptides. For the leaves, the lowest and highest contents of amino acids were found under drought and flooding stresses, respectively; whereas for the roots, the lowest rates were found under drought stress. Gabiroba plants are tolerant to drought stress and remobilize soluble carbohydrates from the shoots to the roots.

RESUMO: O objetivo deste trabalho foi avaliar a tolerância da gabirobeira sob estresse hídrico e alagamento durante 0, 3, 6, 9, 12, 15 e 18 dias. Foram determinados, periodicamente, o conteúdo de matéria seca, água, atividade da redutase do nitrato, açúcar solúvel total, sacarose, amido, proteína insolúvel e solúvel, e aminoácidos solúveis, em folhas e raízes. A deficiência hídrica e o alagamento induziram redução significativa no conteúdo de água e de matéria seca em folhas e raízes. Conforme valores de açúcares solúveis totais, sacarose e amido, houve redução na taxa fotosintética, remobilização de amido de reserva e bloqueio no transporte no floema. A deficiência hídrica acarretou redução da síntese de proteínas e acúmulo de aminoácidos. A maior concentração de proteína insolúvel em folhas e raízes de plantas sob alagamento é consequência da síntese de novos polipeptídeos. O menor conteúdo de aminoácidos em folhas foi encontrado sob deficiência hídrica e o maior, em alagamento; em raízes, as taxas foram menores em estresse hídrico. As gabirobeiras são tolerantes à deficiência hídrica e remobilizam carboidratos solúveis e de reserva da parte aérea para as raízes.
1 Introduction

Drought stress is a key factor among crop production limitations. This phenomenon occurs in large cultivable land areas (Nogueira et al., 2001). Soil waterlogging is another factor that affects plant growth and survival in agriculture and ecosystems. Knowledge on the ecosystem responses to flooding and drought stresses is essential to understand how plants respond to alterations of environmental changes (Horchani et al., 2011).

The gabiroba plant (*Campomanesia* spp) is originally from Brazil and abundant in the Brazilian Cerrado (Souza & Lorenzi, 2005). The absence of the culture of this species, associated with the expansion of the agricultural frontier in the Cerrado, has brought increased attention to its preservation. Several studies report that the fruits of the *Campomanesia* spp tree hold great economic potential for the manufacture of jellies, jams and juices, as well as for medicinal use as anti-diarrheal, antiseptic, and anti-inflammatory (Rodrigues & Carvalho, 2001).

Some works have described the gabiroba plant as rustic and easy to cultivate (Scalon et al., 2009), but no reports have confirmed these characteristics so far. Thus there is still a lack of studies demonstrating its responsiveness to mineral nutrition and culture managements or providing information about its physiology.

Native plants variability in responses to environmental stresses is wide, and there is a knowledge gap on these mechanisms, representing a promising study area to understand the way species adapt to climate changes. This information could broaden the range of uses of natural resources, in addition to serving as a tool for the yield, physiology and molecular biology of this plant, with a view to increasing crop yield.

Plants were classified into three main categories according to the mechanisms developed to withstand drought: species that escape drought (by rapid phenological development); drought-tolerant species with high water potential (postponed dehydration); drought-tolerant species with low water potential (dehydration-tolerant) (Turner, 1997).

Species can adapt metabolically to tolerate anoxia/hypoxia (anoxia-tolerant), adapt morphologically and physiologically to avoid anoxia (apparently tolerant), or fail to adapt and wither rapidly (anoxia-intolerant) (Vartapetian et al., 1978).

The aim of this study was to evaluate the tolerance of Gabiroba plant (*Campomanesia* spp) roots to drought and flooding stresses, aiming at the preservation, management, and domestication of an economically and ecologically important plant species and searching for plant mechanisms that provide flexibility to environmental adversities.

2 Materials and Methods

The experiment was conducted in a greenhouse at the Federal University of Goiás - UFG, Jataí campus, Goias state, Brazil between 10 and 28 February, 2011. A completely randomized experimental design with tree repetitions was used. Eighteen-year-old Gabiroba seedlings (*Campomanesia* spp) were planted in 5 kg pots (one plant per pot) containing Oxisol, previously sieved (<5 mm), with base saturation increased to 60%. Fertilization was applied 64 days after planting, consisting of 55 Kg ha⁻¹ urea (CH₂N₂O), 210 Kg ha⁻¹ triple superphosphate (Ca(H₂PO₄)₂H₂O), and 120 Kg ha⁻¹ potassium chloride (KCl). Soil moisture in the pots was maintained at 70% total pore/volume for 78 days, and the treatments were introduced thereafter.

Plants under drought stress treatment were not watered; plants under flooding stress treatment were in soil waterlogged to 2 cm level of above the surface; the control plants were daily irrigated and maintained at 70% saturation. Drought and flooding stress and control treatments were evaluated for 0, 3, 6, 9, 12, 15, and 18 days in a completely randomized design with three replications.

At the end of each period (0, 3, 6, 9, 12, 15 and 18 days), nitrate reductase enzyme activity was determined in samples of fresh leaves and roots according to the method described by Klepper et al. (1971).

The contents of total soluble sugars, sucrose, amino acids, and soluble protein were determined in an extract from leaves and roots crushed in 10 ml of distilled water, filtered and resuspended in water, completing the volume to 25 mL. For the leaves, the tissue was previously ground in 80% ethanol for chlorophyll solubilization (Bezzerra Neto & Barreto, 2011).

The Yemm & Willis (1954) method was used to determine the total soluble sugars. Sucrose was determined by the anthrone method, with destruction of monosaccharides in the extract by potassium hydroxide (KOH) (Bezzerra-Neto & Barreto, 2011). The α-amino acids were determined by the heating method and α-amino acid with ninhydrin, as proposed by Yemm et al. (1955). Soluble proteins were determined according to Bradford (Bradford, 1976).

To determine starch content, tissue soluble carbohydrates were first removed and starch was hydrolyzed with perchloric acid (52%) and subsequent condensation with anthrone. Starch was determined by multiplying the glucose content by a 0.9 factor. Insoluble protein was calculated between the crude and soluble protein contents (Bezzerra Neto & Barreto, 2011). Contents of dry matter and water in fresh matter of roots and leaves were quantified as described by Silva et al. (2002).

The results were subjected to analysis of variance and means were compared by the Tukey test at 5% probability level, observing the effects of the three treatments for each sampling period.

3 Results and Discussion

Treatments conducted under drought and flooding showed significant decrease in the rates of dry matter for leaves and roots at 12 days compared with the control treatment (Figure 1 - A₁, B₁). These results corroborate the observations by Scalon et al. (2011) on the dry matter of leaves and roots of *Guazuma ulmifolia* cropped on 12.5% of field capacity, as well as those by Figueirôa et al. (2004) on the aerial part and roots of *Myracrodruon urundeuva* cropped on 25% of field capacity.

It is known that drought stressed plants close their stomata to avoid water loss, which consequently decreases photosynthesis, reducing biomass production (Lawlor, 2002). Plants with waterlogged roots also present reduced dry matter production because water-saturated soil blocks water and nutrient uptake by roots and also affect the photosynthetic apparatus, causing...
reduction in photosynthesis and root death (Freitas et al., 2007; Kumar et al., 2012).

In anoxic or hypoxic roots, there is an energy deficit to maintain the physiological processes due to the anaerobic metabolism of roots, contributing little to biomass production. Plants under hypoxia offset this energy deficit through a number of strategies: cytosolic pH control, continuous ATP generation by glycolysis, fermentation, and sufficient storage reserves for anaerobic respiration over long periods and hypoxia or anoxia (Liao & Lin, 2001). In this context, Gregorio et al. (2008) observed a significant decrease on dry matter for Tabebuia heptaphylla, and Costa et al. (2006) observed similar reduction for Schizolobium parahyba. The latter authors also observed high plant death.

![Graphs showing plant growth under different conditions](image-url)

**Figure 1.** Contents of dry matter, water in fresh matter, and nitrate reductase enzyme activity in leaves (A₁, A₂, and A₃, respectively) and roots (B₁, B₂, and B₃, respectively) of Gabiroba plants under drought stress, control treatment, and flooding stress. Means in each period followed by the same letter do not significantly differ by the Tukey test at 5% level of probability.

**Figura 1.** Conteúdo de matéria seca, água na matéria fresca e atividade enzima redutase do nitrito em folhas (A₁, A₂ e A₃, respectivamente) e raízes (B₁, B₂ e B₃, respectivamente) de plantas de Gabiroba sob estresse hídrico, tratamento controle e alagamento. Médias em cada período, seguidas pela mesma letra, não diferem pelo teste Tukey em nível de 5% de probabilidade.
The water content of fresh leaves and roots remained similar to that of the control up to 9 days under drought stress, but it decreased significantly after that (Figure 1 - A, B). Since the changes in relative water content in leaves are small when the stomatal control operates efficiently (Lawlor & Cornic, 2002), this reduction may indicate a defective stomatal regulation to prevent water loss (Kozlowski, 1997).

Under water stress, the osmotic adjustment in the root apex contributes to water accumulation by increasing the turgor pressure of the root cells, targeting growth into wetter soil regions (Taiz & Zeiger, 2009). Two general mechanisms may be responsible for osmotic adjustment in roots: increase of osmoticum or decrease of tissue expansion and, consequently, of the osmoticum dilution rate (Sharp et al., 2004). Lima et al. (2007), studying water deficit in Swietenia macrophylla, reported a reduction in the leaf water potential induced by lower stomata conductance, which affects transpiration and, consequently, decreases the water potential in leaf tissues. According to Lenhard et al. (2010), the relative water content for Caesalpinia ferrea, cropped at 12.5% of field capacity, was not significantly reduced compared with that of the control treatment.

The water content increased to significantly higher values compared with those in the control treatment after 9 days of flooding stress (Figure 1 - A, B). In flooded plants, the relative water content in the cells is very high, because the soil is always above the field capacity, so the plant contains available water even in intercellular spaces, raising the content (Freitas et al., 2007).

Nitrates reductase activity on leaves and roots produced less than 0.25 mMol nitrate in all treatments at 0, 3, 6, 9, and 12 days. The highest values were observed after 15 and 18 days, when the enzyme activity increased (Figure 1 - A, B). This increase in the nitrates reductase activity after 12 days corroborates the results obtained by Oliveira et al. (2011), in which Gabiroba plants presented strong enzymatic activity under drought and control treatments.

Most likely, the increase in nitrate assimilation observed in this study is a particular physiological property of this plant species. To be complexly regulated, the enzymatic activity may have suffered an adaptation with the interaction between nitrate, sucrose, organic acids, and some amino acids, which determines the transcription and translation of the active protein (Kaiser & Huber, 2001).

Drought stress can diminish the enzymatic activity reducing the leaf photosynthetic capacity and the nitrate influx (Helitd, 2005). Hypoxia can also induce nitrogen assimilation. In tomatoes plants, anoxia increased the nitrate reductase activity and dissociated the inhibitory protein 14-3-3, according to Allègre et al. (2004).

Under drought stress treatment, the total soluble sugar content in leaves was similar to that of the control treatment after 15 and 18 days, and was significantly reduced in roots only after three days. Under flooding stress treatment, the soluble sugar content increased significantly in leaves after 15 days and reduced in roots after three days (Figure 2 - A, B).

The lower total soluble sugar contents found for leaves and roots under drought stress were probably due degradation, as the plant metabolism provides alternative ATP production to avoid inhibition of enzymes such as ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), or to limit the photosynthetic rate. Robinson & Portis (1988) showed that Rubisco activity decreases in proportion to ATP concentration in chloroplast stroma. Moreover, under drought stress, root cells absorb soluble carbohydrates to maintain the root system (Sharp et al., 2004).

Although Campomanesia spp has not shown osmotic adjustment to soluble carbohydrate in leaves and roots, Chaves-Filho & Stacciarini-Seraphin (2001) demonstrated an increment of reducing carbohydrates for Solanum lycocarpum. For Lupinus albus, Pinheiro et al. (2001) observed that after 6 days without irrigation, the contents of sucrose, fructose, and glucose increased approximately five times, while sucrose phosphate synthase and acid invertase increased two times.

Several studies have been conducted on osmotic adjustment under hypoxic conditions for different domestic plants. Studying melon plants, Su et al. (1998) observed that the contents of sucrose, fructose, and glucose increased up to four times during fast hypoxic periods, and then gradually decreased. McMannon & Crawford (1971) and Vartapetian et al. (2003) explained that an increase in glycolysis occurs in short hypoxic periods, spending hexoses for energy synthesis.

The total soluble sugar rate in roots and the sucrose content in leaves were significantly reduced after three days of drought stress and hypoxia. Drought stress led Decreased sucrose contents in roots were observed after six days under drought stress, whereas contents similar to those of the control treatment were observed under flooding stress (Figure 2 - A, B).

The low sucrose content in leaves during drought stress periods may have occurred owing to remobilization of starch reserves for interconversion of other carbohydrates and ATP synthesis. Under drought stress, the starch reserve is hydrolyzed by an increase in the amylase activity, resulting in a greater accumulation of soluble carbohydrates, amino acids, and organic acids (Melo et al., 2007).

Silva et al. (2010) also reported a strong reduction in the concentrations of sucrose and starch in leaves of Coffea canephora when submitted to drought stress. Praxedes et al. (2006) explained that the reduction in sucrose concentration under water deficit can occur as a result of decreased synthesis due to increased degradation. It can also occur by enzymatic activity inhibition for sucrose phosphate synthase and increased acid invertase activity.

Under flooding stress, the leaf starch content was significantly increased after 3, 9 and 18 days. In this period, the content observed under drought stress was similar to that found under the control treatment. In roots, starch concentration also increased after 12, 15 and 18 days under flooding stress, but under drought stress it was similar to that of the control treatment (Figure 2 - A, B).

The Gabiroba plant has high starch storage in its roots as energy reserve. This storage ensures the species survival under prolonged hypoxic conditions. Bailey-Serres & Voesenek (2008) observed that amylase induction promotes the conversion of starch into glucose in some plants and tissues with low oxygen concentration; however, starch mobilization during oxygen absence is not universal.

In plants under hypoxia, transport of photosynthates to the phloem or translocation from roots to shoots may have been
Impact of drought and flooding stresses on Gabiroba plants

Insoluble protein contents showed no significant changes in leaves and roots under any of the treatments (Figure 3 - A and B, respectively). In leaves, the soluble protein content reduced significantly after six days of drought and hypoxia, but increased after 12 days of flooding. In roots, there were no significant changes in the soluble protein contents (Figure 3 - A and B, respectively).

Under drought stress, the syntheses of several proteins are inhibited, whereas the syntheses of other proteins that present particular functions in response to drought stress, such as those involved in osmotic adjustment and carbon metabolism, are induced. This leads to an accumulation of soluble sugars in the leaves, which may be used as energy sources or for the synthesis of other compounds.

Liao & Lin (2001) showed that stomatal closure limited CO$_2$ fixation in plants under hypoxia, and that photosynthetic transport to the phloem is blocked, also affecting sucrose storage. This may lead to starch accumulation in the chloroplasts, possibly inducing CO$_2$ fixation reduction by feedback inhibition and starch accumulation. Moreover, there are reports of stomatal closure in plants under hypoxia (Batista et al., 2008), leading to a reduction in the photosynthetic rate.

Figure 2. Contents of total soluble sugar, sucrose, and starch in leaves (A, A and A, respectively) and roots (B, B and B, respectively) of Gabiroba plants under drought stress, control treatment, and flooding stress. Means in each period followed by the same letter do not significantly differ by the Tukey test at 5% level of probability.

Figura 2. Conteúdo de açúcar solúvel total, sacarose e amido em folhas (A, A e A, respectivamente) e raízes (B, B e B, respectivamente) de plantas de Gabiroba sob estresse hídrico, tratamento controle e alagamento. Médias em cada período, seguidas pela mesma letra, não diferem pelo teste Tukey em nível de 5% de probabilidade.
As seen in Gabiroba plants, reduction in protein content due to drought stress may be attributed to increased protein synthesis rate from hydrolysis, reducing the availability of amino acids or enzyme denaturation involved in stress response. El-Tayeb & Ahmed (2007) verified an increase in protein content in apoplastic leaves of drought-tolerant Vicia faba, and a decrease in this protein content for the drought-sensitive type.

Figure 3. Contents of insoluble and soluble proteins, and amino acids in leaves (A<sub>1</sub>, A<sub>2</sub>, and A<sub>3</sub>, respectively) and roots (B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub>, respectively) of Gabiroba plants under drought stress, control treatment, and flooding stress. Means in each period followed by the same letter do not significantly differ by the Tukey test at 5% level of probability.

As dehydrins and aquaporins (Deleu et al., 1999), increase. Mansour (2000) described the accumulation of soluble proteins during drought stress, which was not observed in this study. According to that author, this accumulation occurs due to the maintenance of nitrogen resource to be reused by the plant at the end of drought stress. These proteins may be resynthesized or have an expressive increase in response to stress.

Figure 3. Conteúdo de proteína insolúvel, solúvel e aminoácidos em folhas (A<sub>1</sub>, A<sub>2</sub>, e A<sub>3</sub>, respectivamente) e raízes (B<sub>1</sub>, B<sub>2</sub>, e B<sub>3</sub>, respectivamente) de plantas de Gabiroba sob estresse hídrico, tratamento controle e alagamento. Médias em cada período, seguidas pela mesma letra, não diferem pelo teste Tukey em nível de 5% de probabilidade.
Under anoxia or hypoxia, protein synthesis is modified, the syntheses of some proteins are increased, while others are reduced or even inhibited. The proteins with increased and decreased or inhibited syntheses are related to aerobic and anaerobic metabolism, respectively (Lopes et al., 2005). Most likely, the protein with high molecular weight is linked to the mechanism of hypoxic tolerance; according to Lenhard et al. (2010), the content of nitrogen in the *Caesalpinia ferrea* plant was negatively affected.

After 9 and 12 days under drought stress, the amino acid content in leaves was still similar to that of the control treatment, and higher under flooding stress. The amino acid content in roots under drought stress was significantly reduced after 9 days, whereas after 12 and 18 days, the content was similar to that of the control treatment. After 12 and 18 days of flooding stress, the amino acid content in roots increased significantly (Figure 3 - A, B).

Several osmoprotectants, proline and glycine betaine, are produced in response to drought stress. Proline was identified as an important osmoprotectant in drought-stressed plants (Sofo et al., 2004). Other substances such as betaines, consisting of amino acids such as glycine, alanine and betaine with the methylated amino group, confer protection against drought and salinity stress and also participate as antioxidants in the elimination of reactive oxygen species (ROS) (Heldt, 2005). Silva et al. (2010) observed significant increase in the contents of amino acids and proline for *Coffea canephora* clones submitted to critical drought. According to Taiz & Zeiger (2009), the accumulation of proline and other amino acids can contribute to osmotic adjustment, minimizing the impact of drought on plants.

In hypoxic roots, increased in amino acid content may occur due to proteolysis or interconversion of amino acids, which may be related to the production of a polyamine such as putrescine, formed by decarboxylation of arginine (Reggiani, 1999). Alanine has been frequently reported in hypoxic tissues, and its synthesis is derived from pyruvate formed in large quantities by the acceleration of glycolysis in roots, or by the interconversion of other amino acids (Sousa & Sodek, 2002).

**4 Conclusions**

Mechanisms of tolerance to drought stress observed in Gabiroba plants consist of soluble carbohydrates and remobilization of reserves from the shoots to the roots, thus sustaining root growth into wetter soil regions.

Gabiroba plants sustain the leaf water potential with soluble carbohydrates as a strategy to overcome flooding stress. Most likely, the shift from aerobic to anaerobic metabolism induced by flooding stress leads to synthesis and degradation of polypeptides, causing accumulations of amino acids that contribute to plant survival in case of prolonged hypoxia.

**References**


Author’s contribution: The work is part of the first author’s dissertation, Emiliane dos Santos Belo, who contributed in all stages of construction of this article. Antonio Paulino da Costa Netto contributed as a guide in conducting the experiment and during the biochemical analysis, results analysis and scientific writing. Vanessa Cristina Stein collaborated in the application of laboratory methodologies and text translation. Samuel Mariano Gislon da Silva participated in the biochemical analysis and statistics and scientific writing.

Acknowledgements: At the Federal University of Goiás, Jataí Campus, through the Program of Graduate Studies in Agronomy, which provided location and laboratories to conduct the experiment. To CAPES (Higher Education Personnel Improvement Coordination) by granting scholarship to the first author. At Larissa Vilela Assis ‘in memoriam’, who shared with all authors joy, dedication and support during key stages of the experiment and laboratory analysis.

Funding source: To CAPES (Higher Education Personnel Improvement Coordination) for providing post-graduate scholarship to the first author. CNPQ (National Council for Scientific and Technological Development) for the financing and promotion of research. The Graduate Program in Agronomy of the Federal University of Goiás, which provided a greenhouse, materials and laboratories.

Conflict of interests: The authors declare no conflicts of interest.