Gas exchange and water use efficiency in seedlings of tree species from caatinga under water levels and potassium

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ABSTRACT

This study aimed to evaluate the effects of water availability and potassium on physiological aspects of Myracrodruon urundeuva, Libidibia ferrea, and Mimosa tenuiflora seedlings. The experiment was conducted in a completely randomized design, factorial scheme 3 x 3, with three potassium doses [0, 97.5 and 195 mg dm$^{-3}$ K] and three water levels [100%cv (control), 50%cv (moderate deficit) and 25%cv (severe deficit)]. The plants were kept in plastic bags containing 5 kg of substrate. Relative water content (RWC), transpiration (E), stomatal conductance (gs), net photosynthesis (A) and intrinsic water use efficiency (WUEi) were evaluated. The water deficit did not affect the RWC of L. ferrea and M. tenuiflora seedlings, but caused changes in the gas exchange in the species studied. During the initial phase of growth, M. urundeuva, L. ferrea and M. tenuiflora seedlings can be kept under conditions of low water availability in the soil. The M. urundeuva seedlings showed greater WUEi, even under severe water deficit. The use of K made it possible to maintain high E and A values, being able to be used to mitigate the effects of the water deficit, and we recommended using 195 mg dm$^{-3}$ K.

Keywords: Anacardiaceae. Fabaceae. Mineral nutrition. Stomatal behavior. Water deficit.

RESUMO

Este estudo teve como objetivo avaliar os efeitos da disponibilidade hídrica e da adubação potássica nos aspectos fisiológicos em mudas de Myracrodruon urundeuva, Libidibia ferrea e Mimosa tenuiflora. O experimento foi conduzido em delineamento inteiramente casualizado (DIC), esquema fatorial 3 x 3, correspondendo doses de potássio [0, 97,5 e 195 mg dm$^{-3}$ K] e níveis de água [100% cv (controle), 50% cv (déficit moderado) e 25% cv (déficit severo)]. As plantas foram mantidas em sacos plásticos contendo 5 kg de substrato. Foram avaliados o teor relativo de água (TRA), transpiração (E), condutância estomática (gs), fotossíntese líquida (A) e a eficiência intrínseca no uso da água (EUAI). O déficit hídrico não afetou o TRA em L. ferrea e M. tenuiflora, mas afetou as trocas gasosas das plantas. As mudas de M. urundeuva, L. ferrea e M. tenuiflora podem ser mantidas em condições de baixa disponibilidade de água no solo. As mudas de M. urundeuva apresentaram maior EUAI mesmo sob severo déficit hídrico. O uso de K possibilitou manter altos valores de E e A, podendo ser utilizado para mitigar os efeitos do déficit hídrico durante a fase inicial de crescimento nas espécies avaliadas. Recomenda-se o uso de 195 mg dm$^{-3}$ K.

1 Introduction

Experts have stated that warming rates and climate change are likely to increase in the coming years, predicting an increase in global temperature, leading to increases in drought periods and cycles (IPCC, 2014). These climate changes have been the focus of many studies (FU et al., 2013; SILVA, et al. 2017; ZEMP et al., 2017; CIEMER et al., 2019), and such changes may result in different responses to each group of plant species.

In the Brazilian semiarid region, the problems related to the limitation of water resources are constant due to reduced precipitation, high rates of transpiration and long periods of drought (ARAÚJO, 2011). In addition, the predominance of poorly developed, stony soils with low water retention capacity (ALVES; ARAÚJO; NASCIMENTO, 2009; ARAÚJO, 2011), and in some cases compacted by trampling animals, constitute another challenge for plant growth. The sum of these factors hinders the growth and establishment of forest species in projects or actions for forest restoration and recovery of degraded areas, contributing to the worsening of environmental problems.

Water is the most limiting factor for plant productivity as it is essential for maintaining turgor and consequent cell elongation, the transport of substances in the plant and for gas exchange between leaves and the external environment, in addition to other important metabolic processes of plants, mainly during the initial period of development (SOUZA; SOARES; REGINA, 2001; KERBAUY, 2013; TAIZ; ZEIGER, 2013).

From a physiological point of view, plants have mechanisms that control water loss in situations of water restriction, such as stomatal closure (REICH, 2014), as a survival strategy. The partial closure of stomata aims to decrease the rate of transpiration, maintain leaf water balance and prevent tissue death from dehydration (PEAK et al., 2004), however it limits the assimilation of CO$_2$, reducing the photosynthesis (PEEVA et al., 2009; HU; WANG; HUANG, 2010) leading to death. This stomatal control mechanism has been reported in many studies (CAKMAK, 2005; JIN et al., 2011; MENDES et al., 2013; WANG et al., 2013; SHABALA; POTTOSIN, 2014) and is associated with the potassium ions absorption by the guard cells (MARENCO; LOPES, 2011). This ions accumulation promotes changes in the osmotic potential of cells, leading to water absorption and, consequently, changes in turgor pressure, resulting in the opening of stomata (SILVEIRA, 2000; MENDES et al., 2013).

In addition to ions accumulation like K$, plants are able to perform osmotic regulation through the accumulation of solutes such as sugar (COSTA et al., 2015), amino acids (OLIVEIRA et al., 2017), and proteins (SILVA, 2011), this mechanism has often been highlighted for being an efficient strategy developed by plants to withstand the effects of water reduction. This accumulation of solutes aims to decrease the water potential of the stomata and thus cause the absorption of water from adjacent cells (KERBAUY, 2013), once this process is triggered the plant maintains the necessary cell turgor for the stomata to remain open and perform its physiological processes even in situations where the availability of water is reduced.

In order to minimize the effects of water deficiency on plants, mainly due to the current global scenario, it is necessary that strategies are adopted, aiming to increase the tolerance of plants to this adverse condition. One of the alternatives is the use of potassium (K$) in plant nutrition and the role of this ion in plant resistance to water deficit has been known for a long time (MARTINEAU et al., 2017). Although studies have already pointed out the effects of potassium fertilization on acclimatization to drought, most of these have been developed with agricultural species, and research with this scope in forest species is still incipient and is mostly reported mainly for species of the genus Eucalyptus (SILVEIRA, 2000; MENDES et al., 2013; CHRISTINA et al., 2015; FRANCO, 2018).

* Mimosa tenuiflora* (Wildl) Poiret, from the Fabaceae family, is native to the Caatinga, used for energy purposes (AZEVEDO et al., 2012), forage (BAKKE et al., 2007; ARAÚJO FILHO, 2013), in addition to presenting medicinal properties (BEZERRA et al., 2011; ROQUE; LOIOLA, 2013) and because it is a pioneer species, it has the ability to prepare the soil for the colonization of more demanding species (MAIA, 2004; AZEVEDO et al., 2012). *Libidibia ferrea* (Mart. Ex Tul), also from the Fabaceae family, is a colonizing species of secondary stages, presents wood with high density (VITORIO, 2013), being widely used in civil construction and as energy (LORENZI, 1992; CARVALHO, 2003), for forage (ARAÚJO FILHO, 2013) and landscape resources (NOGUEIRA et al., 2010), with medicinal potential (OLIVEIRA et al., 2010; PEREIRA JÚNIOR et al., 2014), being indicated for restoration of riparian forests (JESUS et al., 2017). The *Myracrodruon urundeuva* Fr. Allemão, Anacardiaceae, colonizer of late secondary stages (LORENZI, 1992), has high resistance wood (VITORIO, 2013), which configures its use for external works, manufacture...
of piles, posts, beams, wood and coal (MAIA, 2004), in addition to presenting pharmacological potential (LUCENA et al., 2011; RIBEIRO et al., 2014; PEREIRA JÚNIOR et al., 2014).

Studies of the effect of water deficit on the physiological and biochemical responses of plants in Caatinga are limited, and there are no studies comparing species belonging to different successional groups, as well as those developed to evaluate the contribution of K+ in mitigating these effects. Thus, this study aimed to evaluate the effects of water availability on the stomatal behavior of Myracrodruon urundeuva Fr. Allem, Libidibia ferrea (Mart. ex Tul), and Mimosa tenuiflora (Willd) Poiret seedlings, and to analyze the role of K+ in mitigating these effects. These species belong to different successional groups, classified, respectively, as pioneer, initial secondary and late secondary, being, therefore, different regarding the water requirement.

2 Materials and methods

2.1 Plant material and growth conditions

The experiment was carried out in a greenhouse at the Forest Nursery of Rural Health and Technology Center, Federal University of Campina Grande (UFCG), Patos, Paraiba State, Brazil (7°03'34" S and 37°16'30" O).

The M. urundeuva, L. ferrea, and M. tenuiflora seeds were obtained from plants kept on the UFCG/Patos Campus. To obtain the seedlings, initially seeds of L. ferrea, and M. tenuiflora were submitted to the overcome dormancy process. The break of the tegumentary dormancy of the seeds of M. tenuiflora was accomplished by immersion in hot water (85°C) for 30 seconds and later washing in running water for one minute (BAKKE et al., 2006). To overcome dormancy in L. ferrea seeds, they were immersed in concentrated sulfuric acid for 30 minutes. After that time, the seeds were washed with distilled water for three consecutive times for two minutes, to remove excess acid.

The seeds were placed to germinate in black plastic bags (28cm x 15cm) containing 4.8kg of substrate (subsoil soil 0 to 20cm), and the fertilization was carried out with N and P according Furtini Neto et al. (1999) recommendations, while the supply of K was carried out according to the treatments tested.

The average emergence time was 12 days, and 15 days after (DAE), thinning was carried out, leaving the plant more vigorous. The irrigation was carried out once a day, trying to keep the substrate humidity close to 100%cp (capacity of substrate retention or pot capacity), determined by weighing. For this, five containers containing the aforementioned substrate were submerged in a bucket of water, until the flooding was noticed. Then, they were removed for complete leaching and then subjected to weighing. The obtained value corresponds to 100%cp and, based on it, the evaluated water levels were determined.

2.2 Experimental design and treatments

The treatments were distributed in a completely randomized design, factorial scheme 3 x 3, corresponding to three potassium doses [0, 97.5 and 195 mg dm$^{-3}$ K] and three water levels [100% cp (control), 50%cp (moderate deficit) and 25%cp (severe deficit)], with potassium chloride (KCl) as the nutrient source, with four replicates and two plants per plot. Water regimes started 30 days after thinning (45 DAE).

2.3 Parameters

a) Stomatal parameters: at 60 days after the beginning of water regimes, using the portable photosynthesis analyzer LCpro-SD (ADC BioScientific Ltd.), the transpiration ($E$), stomatal conductance ($g_s$) and net photosynthesis ($A$). These readings were performed on fully expanded leaves inserted in the second node from the apex of the plants, between 10:00 and 11:00 am. Intrinsic water use efficiency ($WUE_i$) was obtained by the ratio $A/g_s$. The photosynthetically active radiation (PAR) of the equipment was adjusted to 1200 µmol m$^{-2}$ s$^{-1}$.

b) Relative water content (RWC): after stomatal evaluations, four leaves per repetition were harvested and taken to the laboratory, subjected to weighing to determine the fresh weight (FW). Then, they were immersed in water, and under a sheet of filter paper, placed in Petri dishes, and kept in the refrigerator (5°C) for 72 hours. After this period, they were removed, dried on absorbent paper and weighed, to obtain the turgid weight (TW). Subsequently, they were dried in an oven at 65°C for 72 hours, and subjected to weighing to determine the dry weight (DW). RWC was calculated according to equation 1 (WEATHERLEY, 1950).

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$ (Equation 1)
2.4 Statistical analysis

The data were subjected to analysis of variance and the means compared by the Tukey test, at the level of 5% probability, using the software SISVAR version 5.6 (FERREIRA, 2011).

3 Results and discussion

3.1 Relative water content

There was a significant effect of the interaction of treatments on the RWC only in *M. urundeuva* (Figure 1), with no significant difference being detected between the fertilization treatments, in the plants maintained at 100%cp. At 50%cp, the plants that received 195 mg dm\(^{-3}\) K showed lower RWC than the other potassium treatments. In contrast, at 25%cp, the supply of this amount of K to plants provided the maintenance of RWC higher than the other levels of this nutrient, evidencing its positive effect on plants, when under low water availability.

As shown in Figure 1, K did not influence the RWC of *M. urundeuva*, when kept under a high level of water in the substrate (100%cp). However, its beneficial effect was noted under moderate (50%cp) and severe (25%cp) water deficits, in which the highest RWC values were obtained with 97.5 mg dm\(^{-3}\) K and 195 mg dm\(^{-3}\) K, respectively.

The maintenance of high RWC may be due to the fact that K nutrition helps plants in drought tolerance, especially by favoring photosynthesis and the transport of carbohydrates from the leaves to the roots (MARTINEAU et al., 2017a). This, associated with the possible accumulation of K in the roots, may have had an osmotic effect on the cell vacuole, favoring water absorption (PREMACHANDRA et al., 1992). In addition, the accumulation of soluble sugars in plants, together with other physiological mechanisms such as reduction in gs and E, contribute to tissue hydration (LEITE et al., 2018).

Assessing water stress in *Jatropha curcas* L., a semiarid regions species, Sapeta et al. (2013) found higher values of RWC in plants subjected to severe stress, which remained around 83%. Ramos and Freire (2019) observed RWC reducing from 88% in the rainy season to 51% in the dry season, in *Cnidoscolus quercifolius* Pohl.

3.2 Gas exchange

The transpiration (E) of *M. urundeuva* decreased with the reduction in water availability, in treatments 0 and 97.5 mg dm\(^{-3}\) K (Figure 2A); while in plants that received 195 mg dm\(^{-3}\) K, there was an increase with a reduction in the amount of water supplied.

Figure 1 – Relative water content (RWC) of *Myracrodruon urundeuva* (A), *Libidibia ferrea* (B), and *Mimosa tenuiflora* (C) seedlings submitted to water levels and potassium fertilization, during 60 days.

Capital letters compare K doses, within each water regime; lowercase letters compare water regimes in each dose of K.
In *L. ferrea* (Figure 2B) at 100%cp, the addition of 97.5 and 195 mg dm\(^{-3}\) K promoted an increase of 12% and 25%, respectively in \(E\), while in those maintained under severe stress (25%cp), increased by 11% and 19%, showing the positive effect of potassium fertilization on these plants when under low water availability. In *M. tenuiflora* (Figure 2C), there was a significant difference between K doses only in 100%cp treatment. However, plants at 25%cp, in the three K treatments, showed the highest transpiration.

Decrease in the water level promoted a decrease in \(g_s\) of *M. urundeuva* in all K treatments (Figure 3A). However, there was an increase with the progressive increase in the amount of K, in plants at 100%cp and 50%cp.

In 25%cp, the supply of 97.5 and 195 mg dm\(^{-3}\) K provided increases of 186% and 90% in stomatal conductance. Comparing the species, the *L. ferrea* (Figure 3B) showed the highest values of \(g_s\) and, similar to what was found in *M. urundeuva*, the stomatal conductance decreased as the level of water deficit increased, in the plants under treatments 0 and 97.5 mg dm\(^{-3}\) K. In those where 195 mg dm\(^{-3}\) K was added, the highest value of \(g_s\) was found in plants without water deficit (100%cp), followed by plants under severe (25%cp) and moderate water deficits (50%cp).

In *M. tenuiflora* (Figure 3C), plants maintained at 50%cp and which did not receive K, showed the highest \(g_s\) value, with a decrease as the amount of K supplied increased. In contrast, at 100%cp and 25%cp there was an increase in \(g_s\), with an increase in the amount of K.

Analyzing the treatments separately, it appears that within the water regime, the net photosynthesis (\(A\)) of *M. urundeuva* (Figure 4A) decreased when water availability decreased, in all K doses. In those that did not receive K, \(A\) was reduced by half when they were subjected to severe water deficit, compared to well-irrigated plants. As for the interaction between treatments, there is an increase in \(A\) as the dose of K increased. In *L. ferrea* (Figure 4B) and *M. tenuiflora* (Figure 4C), the values varied, with an increase in \(A\) with an increase in K, in plants maintained at 100%cp and 25%cp, and a reduction in those maintained at 50%cp.
Figure 3 – Stomatal conductance ($g_s$) of Myracrodruon urundeuva (A), Libidibia ferrea (B), and Mimosa tenuiflora (C) seedlings submitted to water levels and potassium fertilization, during 60 days.

Figure 4 – Net photosynthesis ($A$) of Myracrodruon urundeuva (A), Libidibia ferrea (B), and Mimosa tenuiflora (C) seedlings submitted to water levels and potassium fertilization, during 60 days.

Under water deficit, stomatal regulation is a strategy to limit water loss by plants (CHAVES et al., 2002). Stomata are responsible for gaseous exchanges between plants and the external environment, controlling water loss through transpiration (SCALON et al., 2011) and playing a central role in fixing atmospheric CO2, while preventing dehydration of tissues and consequent death of the plant (CHAVES et al., 2016).
Many researches demonstrate the direct relationship between water content in the soil and changes in gas exchange, with a progressive reduction in the rate of photosynthesis, stomatal conductance and the rate of transpiration as the water deficit progresses, such as species such as *Jatropha curcas* (SAPETA et al., 2013), *M. urundeuva* (COSTA et al., 2015), *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (PESOA; FREIRE; COSTA, 2017), *Tachigali vulgaris* L.G. (ATAIĐE et al., 2018), and *Hevea brasiliensis* L. (NASCIMENTO; NASCIMENTO; GONÇALVES, 2019).

Under water stress, stomatal closure occurs, contributing to the reduction of water loss due to transpiration, resulting in decrease in CO₂ absorption, with direct effects on photosynthesis (CHAVES; FLEXAS; PINHEIRO, 2009). The effects of water deficiency on the net photosynthesis rate of plants can be due to stomatal limitations, promoting changes in stomata opening and closing or resistance to the influx of CO₂, causing a reduction in your availability to Rubisco (FLEXAS et al., 2004; JACINTO JÚNIOR et al., 2019) or due to non-stomatal limitations as a result of impaired Rubisco regeneration (FLEXAS; MEDRANO, 2002; CHAVES; FLEXAS; PINHEIRO, 2009). In addition, this stomatal closure due to water stress can cause lower efficiency of photosystem II due to the limited amount of water absorbed, thus compromising the production of ATP and NADPH and, consequently, the fixation of CO₂ and the production of sugars (PINHEIRO; CHAVES, 2011).

It was verified that the water level at 100%cp was harmful to the plants in some evaluated parameters, such as RWC in *M. urundeuva* (Figure 1), E in *L. ferrea* (Figure 2B) and E and gs in *M. tenuiflora* (Figures 2C, 3C) and A in the three species studied (Figure 5). The 100%cp treatment may have caused excess moisture in the substrate, leading to oxygen deficiency and limited water absorption by the roots, thus compromising the physiological aspects of the plants. According to Fernandes (2012) water stress is not caused only when water resources become scarce, but also when they are available beyond what is necessary they can become harmful. In saturated environments, O₂ levels are reduced due to less oxygen diffusion, resulting in less water absorption by plants (OLIVEIRA; GUALTIERE, 2017).

In these conditions, the spaces that were filled with oxygen are occupied by water, thus making the environment anoxic (SCREMIN-DIAS; LORENZ-LEMKE; OLIVEIRA, 2011). Decreases in the values of A and gs under flooding conditions have been reported in other studies (GONÇALVES et al., 2013; ROSA et al., 2018; QUEIROZ-ALVES et al., 2019; VIDAL et al., 2019). In *Lonchocarpus sericeus* (Poir.) Kunt ex DC. Lira et al. (2013) found that under flooding there was a 48% reduction in A and gs, and that the intercellular CO₂ concentration (Ci) remained high, which led these researchers to infer that changes in gas exchange were associated with factors other than closure stoma, possibly the photochemical step. Likewise, Queiroz-Alves et al. (2019) submitted *Carpotroche brasiliensis* Engl. to 21 days of flooding, and verify reductions of 74% and 87%, respectively, in the values of A and gs. Species sensitive to such conditions may, in addition to undergoing the changes reported above (OLIVEIRA; GUALTIERE, 2017), reduce root growth, leaf expansion, wilting and ultimately senescence (SCALON et al., 2011).

In water levels and potassium fertilization interaction, it was found that the stomatal parameters were positively influenced by K, with an increase in E (Figure 2), in gs (Figure 3) and A (Figure 4), in the studied species. However, the beneficial effect of K became more evident in *M. urundeuva* and *L. ferrea* plants under severe water deficit.

Possibly K has favored the opening of stomata due to reducing the osmotic potential of the guard cells, resulting in the entry of water and increased turgor (TAIZ; ZEIGER, 2013), favoring gas exchange under conditions of less water availability. Several studies report the central role of K in important functions which contribute to mitigate the effects caused by drought (CAKMAK, 2005; MENDES et al., 2013; DIAS et al., 2019). This ion is essential in maintaining cell turgor and osmoregulation (FOURNIER et al., 2005; MARENCO; LOPES, 2011; BATTIE-LACLAU et al., 2014), stomatal regulation (ARQUERO; BARRANCO; BENLOCH, 2006; BENLOCH-González et al., 2008; PASQUINI; SANTIAGO, 2011; ZAHOOR et al., 2017) and elimination of reactive oxygen species (ROS), which ensures the maintenance of photosynthetically active leaf area under water stress (CHRISTINA et al., 2015). Positive effects of K fertilization have been reported by Mendes et al. (2013) that, evaluating the physiological responses of five genotypes of *Eucalyptusgrandis* x *E. urophylla* to water availability and potassium fertilization, found that genotypes under water deficiency and that received fertilization with K showed an increase in physiological performance with elevation in A and E. Similarly, Ramos and Freire (2019) in *C. queretulifolius* under water deficit verify an increase in E, gs, and A as the doses of K were increased.
3.3 Intrinsic water use efficiency (WUEi)

There was variation between water treatments and potassium fertilization in WUEi of *Myracrodruon urundeuva* (Figure 5A), the highest value being obtained at 25%cp and which received 195 mg dm⁻³ K. In *Libidibia ferrea* (Figure 5B) and *Mimosa tenuiflora* (Figure 5C) no statistical difference was detected between K doses, at 100%cp, increasing with an increase in K, at 50%cp, and reduction at 25%cp. However, in all fertilization treatments, the highest WUEi values occurred in plants maintained under severe water deficit.

The K favored WUEi of *M. urundeuva* under severe water deficit (25%cp) (Figure 5A), and in *L. ferrea* and *M. tenuiflora* (Figures 5B, 5C), under moderate water deficit (50%cp), indicating that even under reduced water availability, the plants were able to fix the carbon necessary to maintain their physiological activities (CHAVES; MAROCO; PEREIRA, 2003). This increase may have been due to the greater positive effect of K in A (Figure 4) to the detriment of gs (Figure 3), in agreement with the information of Tsonev et al. (2011), who reported that a reduction in gs, and not in A, may explain the higher WUEi values of plants under water deficit. According to Chaves, Flexas and Pinheiro (2009), under water stress a small decline in gs can have a protective effect against water stress, as it allows plants to save water and thus improve efficiency in its use.

Martineau et al. (2017b) stated that the results reported in the literature about the impacts of K on WUEi are less clear, probably due to the heterogeneity of experimental parameters, such as age and K status of the plants used, and the diversity of species tested. According to Medrano, Flexas and Galmés (2009), this behavior can vary between species and between different phenophases, however plants that respond to water stress with high efficiency in the use of water can resist drought with better performance.
4 Conclusions

1- The water deficit did not affect the water status of *L. ferrea* and *M. tenuiflora* seedlings, but caused changes in the gas exchange in the species studied, and the magnitude of the responses varied with the species.

2- The *M. urundeuva* seedlings showed greater water use efficiency, even under severe water deficit.

3- During the initial phase of growth, *M. urundeuva, L. ferrea* and *M. tenuiflora* seedlings can be kept under conditions of low water availability in the soil.

4- It is recommended to use potassium during the initial growth of the seedlings as it allows reducing the effects of water deficit and increasing the efficiency of water use, essential during the seedlings establishment phase in the field during vegetation restoration processes.

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