

Effects of Water Deficiency on Plants of *Acacia mangium* Willd

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Abstract—The water deficit causes direct effects on the physiology of plants, affecting the biochemical reactions which results in reduction or stoppage of growth. The present study aimed to evaluate the influence of water deficit on gas exchange, water relations, accumulation of solute proline and activity of antioxidant enzymes in seedlings of *Acacia mangium* Willd., the statistical design was completely randomized design (DIC), represented by four water treatments (100; 60; 40 and 30% of field capacity) with 12 repetitions. Were evaluated at gas exchange ratio, leaf water potential, relative water content, activity of antioxidant enzymes, proline, and dry masses of leaves, stem, root and total. Gaseous Exchange in plants of *Acacia mangium* Willd., were influenced by reduced water availability in the soil. Treatment with 30% of field capacity presented greater accumulation of the amino acid proline content. There has been increased activity of antioxidant enzyme catalase in *Acacia mangium* Willd., in the treatment with 30% capacity field. In the production of dry matter to the most sensitive to water deficiency was the dry mass of leaves and stems, the treatments with 30% and 40% of field capacity.

Keywords—Proline, Climate change, Antioxidant enzymes, Dry.

I. INTRODUCTION

The species of *Acacia mangium* Willd., is one of Australian Acacias widely planted in many parts of the world, has clear benefits in agricultural ecosystems, forestry and agroforestry [1]. The main reasons for the widespread planting of *Acacia mangium* Willd., in mono cultures or in mixed plantations with other species of trees or crops in areas with infertile soil, are your ability to improve soil fertility [2, 3].

In the context of effects of climate change and their changes in ecosystems as low soil water availability, and the average temperature increase on a global scale, are singled out as the main primary causes that can affect negative impact on the productivity of crops worldwide. These abiotic factors compromise and influence particularly physiological processes such as photosynthesis in relation to the use of primary resources such as water, light, temperature, CO₂ and nutrients and, consequently, growth rates [4].

The water deficit causes direct effects on the

physiology of plants, affecting the biochemical reactions which results in reduction or stoppage of growth. On these facts, as with any initiative, many studies and research are required to have a minimum knowledge and consequent economic security of the FMU [5, 6].

The knowledge of species as their answers under the influence of different water conditions is essential, from the early months of growth, when the seedlings are highly susceptible to environmental variations [7]. Therefore, the present study aimed to evaluate the influence of water deficit on gas exchange, water relations, and accumulation of solute proline and activity of antioxidant enzymes in seedlings of *Acacia mangium* Willd.

II. MATERIAL AND METHODS

The experiment was conducted in a protected environment at the Experimental Station of the Federal University of Tocantins, Gurupi Campus, located in the southern region of the State of Tocantins, in altitude of 280 meters in 11° 44 ' 8 " South latitude and 49° 02 ' 58 "

West longitude. The predominant climate in the region, according to Thornthwaite, is of the C2wA type "a", characterized by a wet sub humid climate, and precipitation between 1,600 to 1,700 mm and average annual temperature of 26° C [8].

The seedlings of *Acacia mangium* Willd., were from seeds collected in selected trees in the experimental field of the Federal University of Tocantins, Gurupi Campus. On the occasion of the installation of the experiment, seeds were subjected to treatment of breaking of dormancy with immersion in water heated to 100° C for 60 seconds. Sowing was done directly in cells with volumetric capacity of 290 cm³, where were sown three seeds per container with 60% of commercial substrate bioflora, 30% land of subsoil collected in Gurupi, TO 10% and sand. Mineral fertilizer coverage was held to 30 days after

germination, using ammonium sulphate + 500 g 400 g of KCL/10000 cells [9]. After 25 days of sowing, the raleio, remaining only a change for cartridge, being that of greater force and better positioning in the container.

When the seedlings have filed approximately 150 days old, with about of 35 cm tall plants were selected uniforms which were transferred to polyethylene vessels with a capacity of 5.5 liters, filled with a mixture of 70% of the land of underground, 10% and 20% of commercial bioflora substrate. The soil collected belongs to the class average texture. The vessels were placed on metal benches of 3.0 x 1.20 x 1.0 m length, width and height, respectively with spacing between the vessels of 30 cm. The correction of acidity and soil nutrients used in the experiment was performed according to soil analysis and in accordance with the technical recommendations of the book fifth Approach [10] (table 1).

Table 1: Analysis of fertility of the substrate used in the experiment in pots in the greenhouse

pH CaCl ₂	P	K ⁺	Ca ⁺²	Mg ⁺²	Al ⁺³	H+Al	B	Cu	Fe	Mn	Zn	C.O	M.O
	(mg/dm ³)	Macronutrients (cmolc/dm ³)					Micronutrients (mg/ dm ³)					dag.Kg	
5,6	11,6	0,28	2,0	1,0	0,0	1,50	0,1	0,4	11	0,7	0,5	1,2	2,0

During the acclimatization period of fifteen days in the greenhouse, all vessels were held at field capacity (100% CC). After this period the differentiation of water treatments: CC-field capacity, 60%, 40% and 30% of field capacity, according to the methodology proposed by [11] with a modification (added water on the surface of the substrate). The gravimetric method for the determination of direct CC was developed from four vessels, containing the equivalent of six kg of dry soil in the greenhouse. In the vessels, the deformed structure soil was moistened until saturation added water on the surface of the substrate after underwent drainage for a period of not less than 28 h, until the total cessation of free drainage, with the surface of the ground covered to avoid evaporation, opportunity in which determined the water content retained. The substrate retained 1.6 liters of water, which matched 100% of field capacity. This value plus the substrate dry weight (6 kg), was considered as control treatment (7, 6 kg). The other treatments were computed by the percentages laid down.

The surfaces of the vessels were covered with an opaque plastic, in order to prevent the loss of water by evaporation of the substrate. To maintain water levels established, the water lost by transpiration was reset every day through the weighing of experimental units (plant + pot + substrate) according to each level of water. To carry out this procedure, we used a scale with a capacity of 15

kg.

The statistical design was completely randomized design (DIC), represented by four water treatments (100; 60; 40 and 30% of field capacity) with 12 repetitions.

Leaf water potential (Ψ_f -MPa) was evaluated in two stages on day 6 and 25° days of the experiment in two times, on before tomorrow (5:00, Ψ_f -maximum-MPa) and noon (12:00, Ψ_f -minimum-MPa). For the determination of Ψ_f , were selected the leaves located in the middle third of the plant, and the equipment used was the Scholander pressure Chamber (SCHOLANDER et al., 1964).

Physiological characteristics evaluated were held daily, in the upper third of the plant, the fourth leaf fully expanded. Using the infrared gas analyzer (IRGA), LI-COR, model LI-6400XT (Portable Photosynthesis System). The evaluations were carried out in the period of July 2017 and the measurements were carried out on the morning of the 09:00 ace 11:00 hours, using four plants per treatment. Simultaneously was registered the relative humidity (RH), air temperature (Tar) with the aid of a digital anemometer.

Gas exchange were measured at a CO₂ concentration of 360 mmol mol⁻¹, temperature of 28° C and photosynthetically active saturated light radiation (PAR) of 1500 mmol m⁻² s⁻¹.

Has been evaluated, the internal carbon (Ci), net CO₂ assimilation, stomata conductance to water vapor (gs),

transpiration rate (E), as well as calculate the efficiency of water use (EUA), obtained by the relationship between amount of CO₂ fixed by photosynthesis and amount of water transpired and carboxylation efficiency (A/Ci).

For biochemical analyses, two collections of leaves, on day 6 and at the end of the experiment, constituting a total of 4 repetitions, packed in liquid nitrogen and stored in the -80 ultrafreezer °C. For subsequent determination of proline and enzyme activity of catalase, superoxide dismutase and ascorbate peroxidase.

The concentration of proline was evaluated by [12]. The activity of the enzyme's catalase, superoxide dismutase and ascorbate peroxidase were determined according to the methodologies adopted by [13] [14, 15], respectively.

The evaluations of dry pasta were measured at the end of the experiment in order to estimate the effects of the treatments on plant development, plants were separated in aerial part and roots, both parties were placed in paper bags, kept in an oven with air circulation ($75 \pm 2^\circ$ C) until reaching constant weight. Then heavy in scale and semi-analytical were evaluated variables: accumulation of dry mass: of leaves (MSF), stem (MSC), roots (MSR) and total dry mass (MST).

The data of the variables leaf water potential (Ψ_f -MPa), relative water Content, activity of antioxidant enzymes, PROLINE and physiological were submitted to descriptive statistics and analysis of variance (ANOVA), and the means were compared by Tukey 5% level of probability using the SISVAR software program [16]. For air temperature variables (Tar), relative humidity (RH) averages were calculated and \pm standard deviation. For the calculation of average values and clothing of the graphs of gas exchange SigmaPlot software 10.0 was used.

III. RESULTS

During the trial period of twenty-five days inside the greenhouse the average values of relative humidity (RH) and air temperature (TEMP) along the experiment were $39.8\% \pm 7.6$ and $32.7^\circ \text{C} \pm 3.0$, with $57.2\% \pm 2.1$ peak

and $38.5^\circ \text{C} \pm 0.6$ and minimum $27.1\% \pm 3.2$ and $27.2^\circ \text{C} \pm 0.8$ respectively.

The average rate values of stomata conductance (gs), net assimilation, transpiration rate (E), instant carboxylation efficiency (a/c) and water use efficiency (EUA) plants of *Acacia mangium* Willd., were reduced with decreased water availability in the soil, showing significant variations over time (Figure 1).

With respect to the variation in stomata conductance (gs) during the trial period, it is observed that plants under 100 and 60% of field capacity (CC) showed the highest values. However, the plants of treatments with 40 and 30% CC presented the lowest values of stomata conductance under water deficiency (Figure 1).

The plants submitted to hydric condition of 100 and 60% CC kept their stomata open over time, leading to greater consumption of water. However, plants under 40% of the CC variation presented the values of stomata conductance, over time, noting a decrease and a slight increase in the feeling of tolerance which reflects the exposure time of the plant under stress hydric. Treatment plants with 30% of field capacity were reduced stomata conductance by water deficit (Figure 1).

For net assimilation rate (A), the largest reductions were observed on water condition of 30 and 40% of the CC to the 5, 15 and 25 days of evaluating plants under 30% CC showed extremely low values, less than zero, suggesting the total outage CO₂ assimilation during this period (Figure 1).

On condition of 30% and 40% field capacity intercellular CO₂ concentration (Ci) was greater than in the control (100% CC), with a net assimilation rate on day 5 to zero both (30% and 40% CC) and at 15° and 25° for plants with 30% CC (Figure 1).

In young plants of *Acacia mangium* Willd., observed a marked recovery rate of stomata conductance (gs), net assimilation, transpiration rate (E), carboxylation efficiency (a/c) and water use efficiency (EUA) for plants subject to the condition that 40% of the CC after the fifth day of water stress (Figure 1).

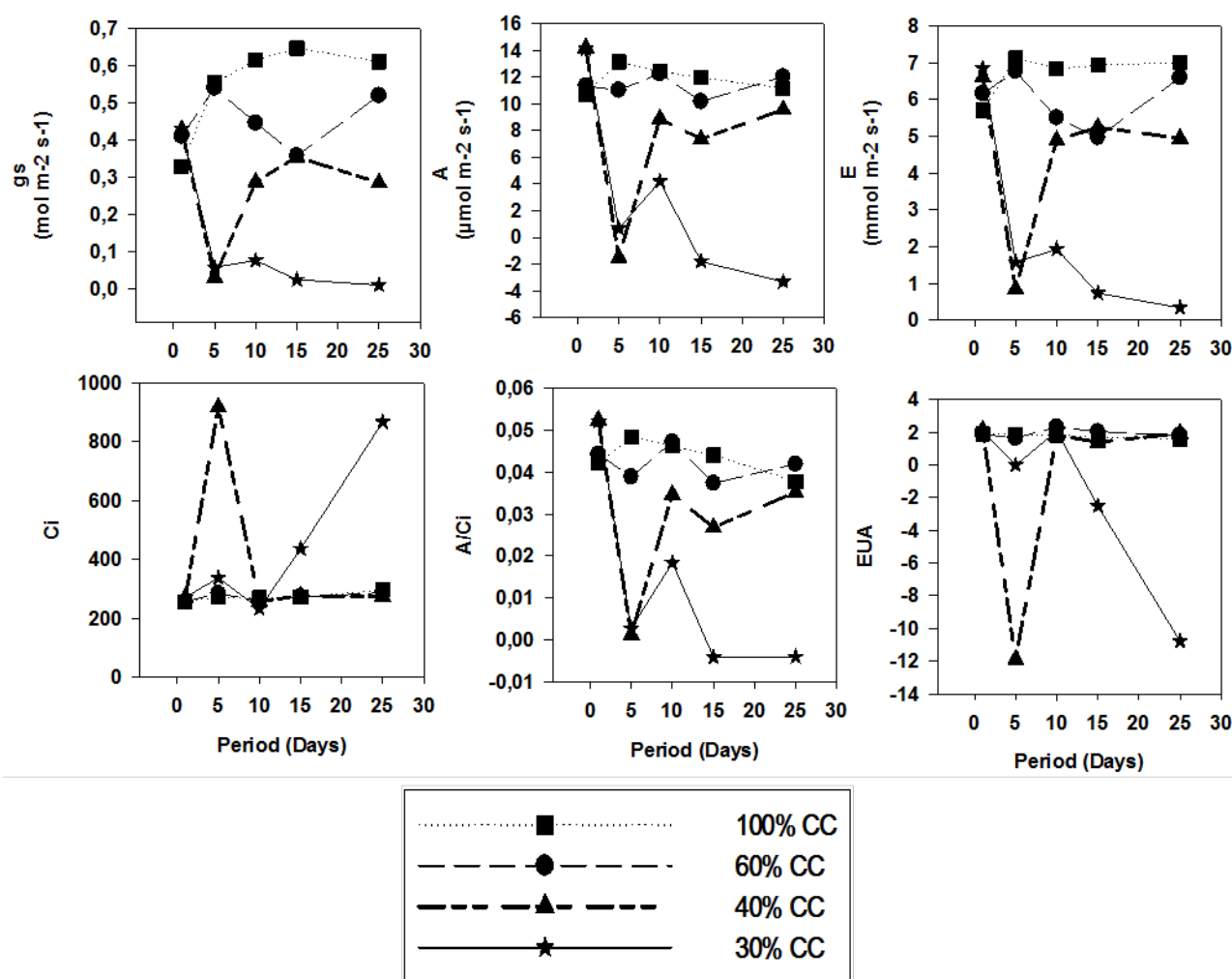


Fig. 1: Stomata conductance (g_s), net CO_2 assimilation rate (A), Transpiration (E), internal CO_2 Concentration (C_i), carboxylation efficiency (a/c) and water use efficiency (EUA) obtained in young plants of *Acacia mangium* Willd., subject to water deficit. Each point represents the average of eight determinations.

For the leaf water potential (Ψ_f -MPa) in *Acacia mangium* Willd., under water deficit at the time of initial evaluation during 5:00 and 12:00 treatment with 60% of field capacity showed no significant change in leaf water potential (Ψ_f -MPa) compared with plants to 100% of the CC, except for the potential measured at the end of the experiment at 12 hours (table 2 and 3).

Plants with 30 and 40% of the CC showed significant reductions in all seasons and times of evaluations, observing in the final period of the experiment in 5 hours and of the 12:00 a more pronounced decrease (-2.85 and -1.83 Mpa respectively) differed from

the other treatments with plants under 60 and 100% of the CC (table 3). Noting that the plants under 30 and 40% of the CC were severely damaged by water deficit.

To 25° days of exposure to water stress plants of *Acacia mangium* Willd., under 100% and 60% CC on time of 5:00 in the morning, the plants under 100% and 60% of the CC presented significantly similar responses showing statistical difference between them. However, differ from plants under 40 and 30% of the CC. At the time of the 12:00, all treatments differed statistically among themselves (table 3).

Table.2: Leaf water potential (Ψ -MPa) in young plants *Acacia mangium* Willd., under water deficit at the beginning of the experiment

Treatments (%CC)	Ψ (MPa)	
	Start (5 Hours)	Start (12 Hours)
100%	-0,15 a	-0,38 a
60%	-0,23 ab	-0,43 ab
40%	-0,28 bc	-0,60 ab
30%	-0,35 c	-0,65 b
CV (%)	14,11	16,40

* Medium followed by letters the same, do not differ statistically between themselves by Tukey test ($P < 0.05$).

Table.3: Leaf water potential (Ψ -MPa) in young plants *Acacia mangium* Willd., under water deficit at the end of the experiment

Treatments (%CC)	Ψ (MPa)	
	End (5 Hours)	End (12 Hours)
100%	-0,30 a	-0,80 a
60%	-0,50 a	-1,30 b
40%	-0,93 b	-1,83 c
30%	-2,0 c	-2,85 d
CV (%)	8,87	3,88

* Medium followed by letters the same, do not differ statistically between themselves by Tukey test ($P < 0.05$).

The proline content to *Acacia mangium* Willd., followed the trend of increased up to 30% conditions of CC differing statistically from the other plants under water condition of 40, 60 and 100% of the CC (Figure 2).

The plants of *Acacia mangium* Willd., when subjected to a greater water stress (30% of field capacity) showed high activity of the enzyme catalase (CAT), being inhibited in witness with 100% of field capacity. Ascorbate peroxidase enzymes (APX) and the superoxide dismutase (SOD) activity remained similar to the control with the increase of water stress conditions. For this species the CAT was the main enzyme in combat the oxidative stress (Figure 2).

The water deficit in plants of *Acacia mangium* Willd., significantly decreased the production of leaf dry mass (MSF) and stem (MSC). Being the dry mass of

leaves of the plant one of the variables most affected by the reduction of the water content in the substrate during the experiment, with the lowest value observed for plants under 30% of field capacity (17.74 g. plant-1) and higher for plants under 100% CC, (33.13 g. plant-1) these being statistically different among themselves (table 4).

To stem dry mass, greater value for plants under 100% CC (21.92 g. plant-1). Plants with 60% and 30% of field capacity showed intermediate values not statistically different from the other treatments (14.87 g. 1 plant and 16.07 g. plant-1 respectively). The lowest value was observed for treatment with 40% of field capacity (13.32 g. plant-1) (table 4).

How to root dry mass and total, the results showed no significant difference for the evaluated treatments (table 4).

Table 4: Dry mass (MS) leaf (F), (C) the stem, (R) and (T) total for seedlings of *Acacia mangium* Willd., under water deficit at the end of the experiment

Treatments (%CC)	MSF (g. plant -1)	MSC (g. plant-1)	MSR (g. plant-1)	MST (g. plant-1)
100%	33,13 a	21,92 a	29,19 a	84,25 a
60%	27,66 ab	14,87 ab	38,15 a	80,68 a
40%	23,11 bc	13,32 b	36,36 a	72,80 a
30%	17,74 c	16,07 ab	39,38 a	73,19 a
CV (%)	14,23	22,30	25,25	16,16

* Medium followed by letters the same, do not differ statistically between themselves by Tukey test ($P < 0.05$).

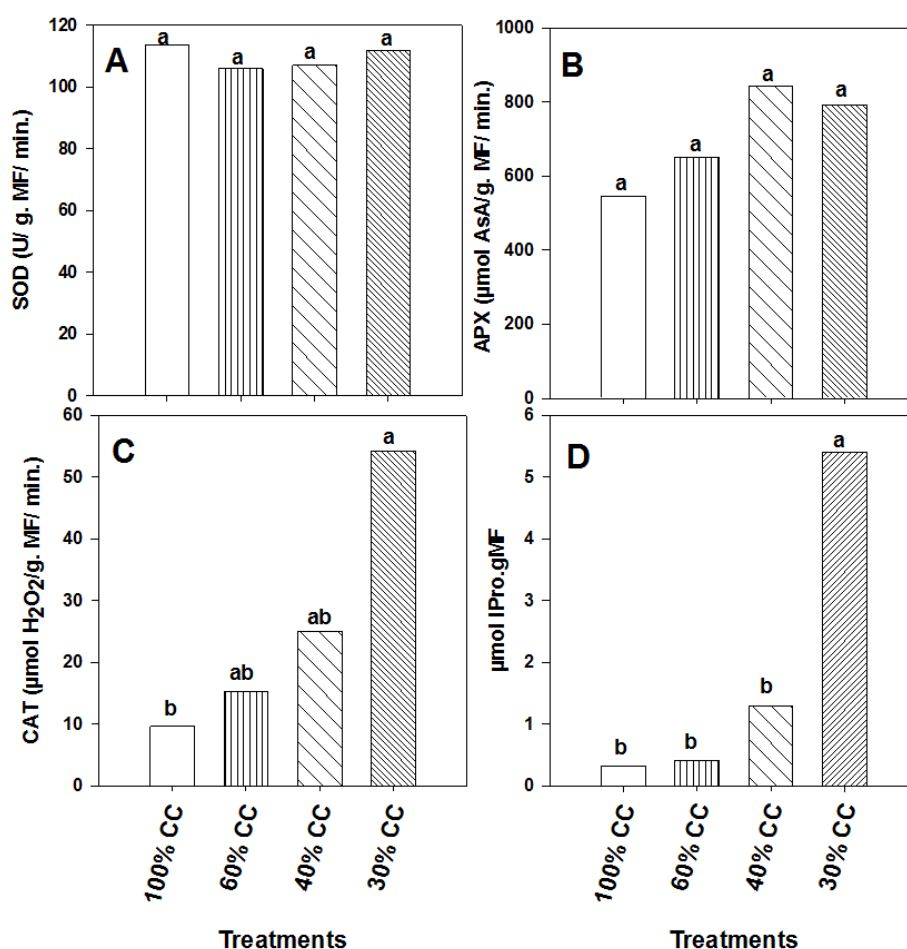


Fig. 2: Activity of antioxidant enzymes (A) superoxide (SOD), (B) peroxide of ascorbate (APX), (C), catalase (CAT), average values of the levels of proline (D) sheets of species of *Acacia mangium* Willd., under water deficit.

IV. DISCUSSION

The water deficit in the soil has caused metabolic changes in different species of plants [17, 18]. However, with the passage of time of exposure in water deficiency conditions, plants can develop certain tolerance while maintaining breath ability for longer periods. In this study it was observed that plants of *Acacia mangium* Willd., when subject to the condition that 40% of field capacity reflects that the affirmative (Figure 1).

It was observed that the closure of the stomata was the first factor of reduction in net assimilation (A), transpiration rate (E), instant carboxylation efficiency (a/c) and water use efficiency (EUA) plants of *Acacia mangium* Willd., under conditions of increased hydric deficit (30% and 40% of the CC). This reduction is due to the decrease in availability of CO₂ in the Chambers of sub estomatic leaves, caused by the closing of stomata (Figure 1) [19].

[20], in your work also noted the decrease in the values of stomata conductance in *Pterogyne nitens*, *Aspidosperma polyneuron* e *Myroxylum peruiferum* when

subjected to different availability of water in the soil.

For water use efficiency (EUA) in plants of *Acacia mangium* Willd., it was observed that under conditions of 100%, 60% and 40% of the CC there was no large variations for the days 10, 15 and 25 days of experiment. Demonstrating a high efficiency and regulation of water use for treatment with 40% field capacity, this being the second most extreme water deficit condition (Figure 1).

The decrease in water use efficiency under the conditions of 30% of field capacity observed in 25 days of experiment can be due to the low availability of water found in the substrate and the time under this condition. Since the conditions of greater availability of water (100%, 60% and 40% field capacity) were not detected variations, observing the highest values (Figure 1).

The intercellular CO₂ concentration low (Ci) under the conditions of 30 and 40% field capacity, evidenced also with null net assimilation rate on some dates evaluated, can be assigned when the stomata conductance decreases, an increase of Ci can be detected. However, as the greater decrease occurs in relative water content, the

CO₂ release photo respiratory source, this is from no photosynthetic fixation becomes relatively high, and a greater Ci can be verified.

Instant carboxylation efficiency (A/Ci) in *Acacia mangium* Willd., along with the closing of stomata (gs), reducing transpiration (E) and net assimilation (A) and the decrease in the availability of water in the soil, reduce your status, by the fact of these plants possibly stop assimilate carbon, due to stomata limitation mainly on treatment with 30% of field capacity (Figure 1).

According to [21], water relations, the water stress acts directly in reduced leaf water potential (Ψ_f -MPa). Corroborating with this statement was evidenced in this study that the 30% CC plants showed significant reductions compared to other water conditions in all seasons and times of evaluations. Noting that the plants under 30% of field capacity has been severely affected by water deficit (table 3).

In this work at the end of the experiment on the 25th day under water stress to 30% of field capacity plants were severely hindered observing during 12:00 a reduction pronounced leaf water potential (-2.85 Mpa) plants differing in the too much water conditions (table 3).

The water stress can cause oxidative stress in plants, due to the increased production of reactive oxygen species (ROS), highly toxic in the living organism. The reactive oxygen species (ROS) as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^{\bullet}) cause damage to proteins and lipids of cell membranes of plants [22, 23].

To combat the production of ROS plants exhibit antioxidant mechanisms including antioxidant enzymes such as superoxide (SOD), ascorbate peroxidase (APX) and catalase (CAT) which prevent oxidative damage [24].

The lowest water availability of 30% of field capacity for seedlings of *Acacia mangium* Willd., caused this species needed antioxidant system, mainly of the enzyme catalase, due to your contribution to this condition. Given this, the probability of occur in cellular metabolism imbalance is high, induced by reactive oxygen species, mainly hydrogen peroxide which is found in high concentrations in plants under this environment.

Results that agreed with [25] that highlighted that the catalase enzyme was elimination of hydrogen peroxide more important for tolerance to stress. Increase in CAT activity under water deficit conditions was also observed in seedlings of *Copaifera langsdorffii* Desf. [7] e *Schinus terebinthifolius* Raddi. [21].

Many vegetables when subjected to abiotic stress change your metabolism. A compound that can be characterized as a drought tolerance factor for the plant is

proline [26]. Proline accumulation in leaves of plants under water deficit conditions has osmoprotect function, acts as composed of carbon and nitrogen stocks during the water deficit, and as anti-oxidant [27].

In this study it was observed that the treatment with the highest water deficit with 30% of field capacity, presented the greater buildup of the amino acid proline content, noting that the water deficit increased statistically this solute concentrations compared to other plants under water stresses (Figure 2). So is regarded as one of the indicators that presents a strong relation to water deficit tolerance in plants of *Acacia mangium* Willd.

These results show that the coordinated action of the South and the enzyme catalase antioxidative proline responded better to face the harshest stresses applied.

The first visible effects of water deficit on plants of *Acacia mangium* Willd., were observed from the fifth day of stress with the appearance of dryness in the leaves (especially older), followed by leaf abscission mainly on treatment with 30% of field capacity.

In this study it was observed that the root development of plants subjected to higher degrees of water deficit (30% and 40% CC) was statistically similar to the control plants (100% of field capacity) and the area of the leaves and stems were statistically smaller for the treatments with higher degrees of water deficit with 30% and 40% field capacity respectively (table 4).

V. CONCLUSION

Gaseous Exchange in plants of *Acacia mangium* Willd., were influenced by reduced water availability in the soil. Treatment with 30% of field capacity presented greater accumulation of the amino acid proline content.

There has been increased activity of antioxidant enzyme catalase in *Acacia mangium* Willd., in the treatment with 30% capacity field.

On dry matter production in seedlings of *Acacia mangium* Willd., the more sensitive the water deficiency was the dry mass of leaves and stems in the treatments with 30% and 40% of field capacity.

The results showed that the *Acacia mangium* Willd., physiological and biochemical changes introduced in an attempt to tolerate the water deficit.

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