Influence of Some Parameters in Seed Germination of *Acacia mangium* **willd**

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Abstract— The objective of this work was to analyze the influence of pH, amount of water on the substrate and salinity on the germination of Acacia mangium Willd seeds. Three experiments were performed. To verify the pH effect, five treatments corresponding to the levels (3.0, 5.0, 7.0, 9.0 and 11.0) were used. As for the amount of water in the substrate, four treatments were evaluated: T1- water quantity 1.5 times the substrate weight (~ 3 ml); T2 - amount of water 2.0 times the weight of the substrate (~4 ml); T3 - amount of water 2.5 times the weight of the

substrate (~ 5 ml) and T4: amount of water 3.0 times the weight of the substrate (~6 ml). In the analysis of the effects of salinity on germination, three aqueous solutions were evaluated: potassium chloride (KCl), calcium chloride (CaCl2) and sodium chloride (NaCl) in 4 concentrations (25, 50, 75 and 100 million mM). All experiments were conducted on four replicates of 50 seeds for each treatment. The amount of water 2.5 times the weight of the substrate leads to higher germination of the acacia seeds, but with increasing salinity, there is a continuous decrease of germination, IVG, and initial growth, mainly with the salts KCl and CaCl2. The pH where the seeds had the best germination corresponded to pH 3.

Keywords—pH; saline stress; water; physiology; biochemistry.

I. INTRODUCTION

Acacia mangium Willd is a fast growing, rustic and tolerant tree species tolerant to a wide range of soil types and environments GRIFFIN (2014). In Brazil, this species is popularly known as acacia, and its wood is usually used in the production of pulp, paper, particle board, and agglomerates, and also presents great potential for the production of sawn wood, alloys, furniture, firewood and charcoal, according to its calorific value (between 4800-4900 Kcal kg-1) KRISNAWATI et al. (2011).

It is of fundamental importance to know the proper conditions for seed germination since these can interfere in this process CARVALHO and NAKAGAWA (2012). Many studies on seed phenology seek methods for the breakdown of dormancy and the influence of environmental factors and conditions that affect the germination process, such as water quantity, salinity and pH, in a recent paper by the University of California, Los Angeles, California, and colleague NUNES et al. (2009); GONÇALVES et al. (2015).

In addition, it is important to note that germination is the most important factor in the germination process in the germination process STEFANELLO et al. ([date unknown]), REGO et al. (2011). When germination tests are carried out in the laboratory, the moisture of the substrate must be maintained uniformly to supply the seeds with the amount of water necessary for germination and development, however, excess moisture can cause a decrease in germination and increase the incidence of fungi, leading to reduced viability FIGLIOLIA and OLIVEIRA (1993).

Salinity is an important parameter to be analyzed for being a stress factor for plants. The increase in salinity causes the reduction of the water potential of the substrate, reducing the gradient between the substrate and the surface of the seed, causing a restriction in the water absorption by the embryo, which, together with the toxic effects of the salts, directly interferes with the germination process of seeds DEMINICIS et al. (2007).

The pH can be another factor that becomes one of the critical points of the germination test, according to the quality system precepts GADOTTI et al. (2013). Many investigations regarding the use of inorganic acids to overcome dormancy have been carried out, but there are few studies about the interference of acids and bases in the germination process CAVALCANTE and de PEREZ (1996). Assumpção and Perine (2016) ASSUMPÇÃO and PERINI (1993) found that the germination potential of S. occidentallis (L.) Link increased significantly when subjected to chemical scarification with sulfuric acid. For this reason, it is significant to carry out a germination test with several pH levels.

The objective of this study was to analyze the influence of pH, water content on the substrate and salinity on the germination of Acacia mangium Willd seeds, in light of the context and the lack of information on the eucalyptology of acacia seed germination.

II. MATERIAL AND METHODS

The present work was carried out at the Laboratory of Seeds of Ecophysiology and Weed Management of the Federal University of Tocantins (UFT), Campus Universitário de Gurupi, located in the southern region of

the state of the Tocantins, lat. 11 $^{\circ}$ 43 'S and long. 49 $^{\circ}$ 04'W, at 280m altitude.

The seeds of Acacia mangium Willd, were collected at the institution (UFT), later transported to the UFT (LSEMPD) of the UFT, Gurupi campus, where they were benefited and manually selected, discarding those that were injurious or deformed.

The number of replicates and number of seeds, the type of substrate, the number of days of evaluation and the overcoming of dormancy was determined according to the instruction manual for analysis of forest species of the Ministry of Agriculture, Livestock and Supply BRASIL (2013).

The seeds were placed to germinate in sterile Petri

dishes on two sheets of germinate paper, weighing 2 g, moistened with the solution according to the treatments of each experiment. Acacia seed dormancy was achieved by

immersing in the water at 100 $^{\circ}$ C for 1 minute.

Three experiments were carried out: the amount of water in the substrate, different levels of pH and saline stress.

Amount of water in the substrate

For this experiment were four treatments: T1 - amount of water 1.5 times the weight of the substrate (~ 3 ml); T2 - amount of water 2.0 times the weight of the substrate (~ 4 ml); T3 - amount of water 2.5 times the weight of the substrate (~ 5 ml) and T4- amount of water 3.0 times the weight of the substrate (~ 6 ml).

Different pH levels

The influence of pH on the germination of *Acacia* mangium Willd seeds was tested under the values of 3.0; 5.0; 7.0; 9.0; and 11.0. To adjust the pH of the above values, 1N sodium hydroxide (NaOH) and hydrochloric acid (HCl) diluted in distilled water were used in an amount sufficient to raise or lower the pH, and it was measured using a pH meter MAYEUX and SCIFRES (1978).

Saline stress

In the analysis of the effects of saline stress aqueous solutions of sodium chloride (NaCl), potassium chloride (KCl) and calcium chloride (CaCl2) and at the concentrations of 25, 50, 75 and 100 millimolar (mM) were prepared.

In all experiments the plates were placed in a BOD incubator with photoperiod 12/12 hours a day/night at 30

 $^{\circ}$ C. The germination evaluations were daily from the 7th day of incubation until the 21st, where the radical and shoot length were measured. The germination was evaluated daily from the seventh day after the installation of the treatments using as criteria the radius protrusion (2 mm). The germination velocities were calculated by the expression:

At where:

$$IVG = \left(\frac{G1}{N1}\right) + \left(\frac{G2}{N2}\right) + \ldots + \left(\frac{Gn}{Nn}\right) (1)$$

G: number of normal seedlings computed at the first count, at the second count,

at the last count.

N: number of days of sowing to the first, to the second to the last count.

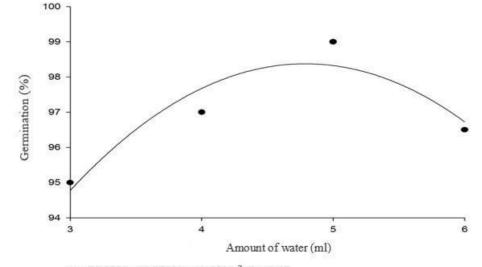
For all the experiments, the experimental design was completely randomized, with four replicates of 50 seeds for each treatment. Each experiment was submitted to regression analysis using Sigmaplot 10.0 software and the regression model chosen was based on the significance of the coefficients of the regression and determination equation at 5% probability.

III. RESULTS AND DISCUSSION

Amount of water in the substrate

The response of the germination percentage of the acacia seeds to the different amounts of water in the substrate (Figure 1) was adjusted to a second-degree regression with a regression coefficient of 0.87. By means of the adjustment curve, it is evident that water levels above 5 ml promote a decrease in the germination of

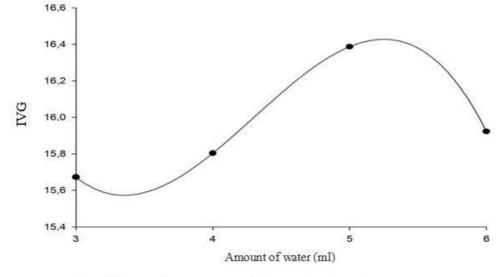
Acacia seeds, although they are still above 90% development. According to Gonçalves et al. (2015) FLORES et al. (2013), excess moisture causes a decrease in germination, since it prevents oxygen penetration and reduces the resulting metabolic process. Despite the reduction observed in the germination in the graph, the data demonstrate that the water levels used were not sufficient to promote drastic reductions in the germination of Acacia seeds, either due to the effect of little or excess water.



 $y = 72,5750 + 10,7750 \times -1,1250 \times^2 R = 0,87$ Fig.1: Germination (%) of Acacia mangium Willd seeds depending on the amount of water in the substrate.

Gonçalves et al. (2015) FLORES et al. (2013) in their experiment with seeds of *Parkia platycephala Benth*, using the same proportions of water as the present study, concluded that there was no interference of moisture in the percentage of germination. Ramos et al. (2006) RAMOS et al. (2006) verified that the amount of water 1.5 the weight of the substrate showed a lower percentage of germination for the *Schizolobium amazonicum* Huber ex Ducke (Paricá) forest, where the best results were using 2.5 and 3.0 times the weight of the substrate (with 85% germination). For the species *Amburana cearensis* (Allemão) AC Smith the highest germination percentage (94%) was obtained with the volume of water of 3.25 times the weight of the dry substrate, however from this volume, the germination was negatively affected GONÇALVES et al. (2015).

As for IVG (Figure 2), the data were adjusted to a cubic curve with R = 0.98, with an increase in velocity with an increase in the amount of water up to 5 ml, after which there was a strong fall. In spite of the observed, the values of the IVG can be considered good, explained by the already mentioned previously as the water levels used in the treatments are not enough to observe more expressive effects in the reduction of the germination of the Acacia seeds.



y = 32,9715 -13,1866*x +3,2226*x² -0,2497*x³ R = 0,98

Fig.2: Germination velocity Index (GVI) of Acacia mangium Willd seeds depending on the amount of water in the substrate.

Flores et al. (2013) FLORES et al. (2013) for the species Melanoxylon brauna Schott (Braunna) presented lower IVG only in the amount of 3.0 times the substrate and was not affected in amounts of 1.5 times the weight of the substrate. Guedes et al. (2010) GUEDES et al. (2010) verified an increase in the IVG with an increasing amount of water used to moisten the substrate.

The length of the aerial part and radical (Figure 3) presented a quadratic behavior with R = 96 and 93, respectively, and the tendency was different to

germination and IVG for the parameter water quantity in the substrate. It is observed that the length of both the aerial part and the radical decreases when there was an increase in moisture, and in the amount of water 1.5 times the weight of the substrate (3ml) there was a larger value of both the aerial part (2, 65 cm) and the radical (2.77 cm), decreasing to the highest amount of water (6 ml). The lowest values for the shoot (1.46 cm) and radical (1.71) were observed.

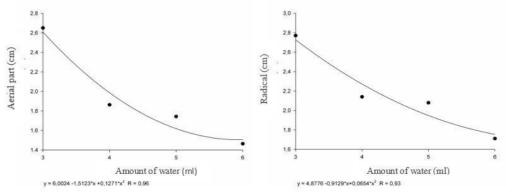


Fig.3: Length of aerial part and radical of Acacia mangium Willd seeds depending on the amount of water in the substrate.

Seed of faveira, which like acacia is a legume, presented different results, wherein the length of the aerial part they maintained a uniform characteristic, presenting an average length of 6.85 cm, but for radical the largest length was verified with volumes of water equal to 3.0 and 3.5 times the substrate weight GONÇALVES et al.(2015).

Varela et al. (2005) VARELA et al. (2005) in its study

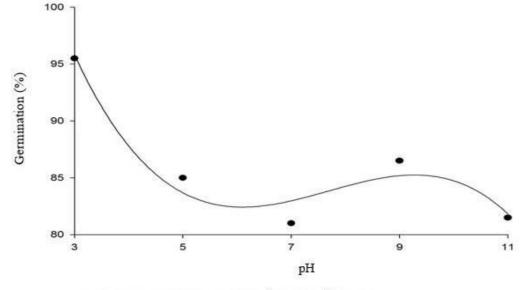
with angelim-stone, also a leguminous, found results similar to acacia, where there was a reduction in root length with the increase of water in the substrate, is that the amount 1.5 times the weight of the substrate presented larger radical lengths and 3.0 times the weight of the lowest substrate.

Different pH levels

The different pH levels promoted differences, that is,

reductions in the germination of Acacia Mangium Willd seeds. from pH 5 (Figure 4). The polynomial regression

analysis showed a quadratic equation of pH levels at germination with R = 0.94.



y = 154,0652 -30,0729*x +4,0848*x² -0,1771*x³ R = 0,94

Fig.4: Germination (%) of Acacia mangium Willd seeds depending on different pH levels.

The species responded to the different variations in pH levels, presenting germination above 80% at all levels. It was observed that the highest percentage (95.5%) of germination was obtained under pH 3 conditions. Regarding the other pH solutions evaluated, germination of pH 5 (85%), 7 (81%), 9 86.5%) and 11 (81.5%). These data indicate greater plasticity for the seeds of *Acacia magium* Willd. ger in pH environments at different levels. In other words, the results indicate that the species has a favorable

ability and adaptation ability to germinate from soils or acid solutions to alkaline BATRA and KUMAR (1993). The IVG presented from the behavior of the regression curve, for the five pHs used

(Figure 5), was similar to the germination curve. It is observed that at pH 3 there was a higher (15.7) IVG and at the more alkaline pH 11 used in the experiment it was the smaller (13,14) IVG.

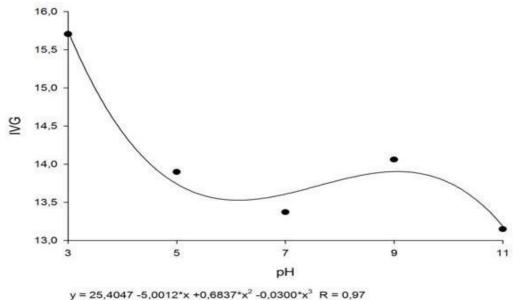


Fig.5: Germination velocity Index (GVI) of Acacia mangium Willd seeds depending on different pH levels.

The length of the aerial part and radical of acacia

(Figure 6) were not influenced by the different pH levels.

The largest length of the shoot was verified at pH 5 (2.49 cm) and the lowest at pH 11 (2.20 cm), with no significant differences between them. The radical presented higher lengths at pH 3 (2.99 cm), decreasing at the other pH's.

These values demonstrate that the species tolerates alkaline and acidic pH, and can be planted in the most diverse types of soil with varying pH.

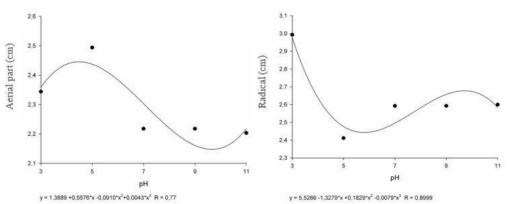


Fig.6: Length of aerial part and radical of Acacia mangium Willd seeds. Depending on different pH levels.

Saline stress

The germination of acacia seeds submitted to different concentrations of salts (Figure 7) was framed in a quadratic equation with R = 0.99. The KCl and CaCl2 showed the same tendency of decrease in the germination (%) with the increase of the concentrations, mainly from 75 mM. The NaCl obtained higher germination in the concentration of 25 mM (98%) and lower in 100 mM (83.5%) as in other salts, but with 50 mM there was a decrease in germination with 88% and in the concentration of 75 mM it increased to 95% germination. The lowest germination salt (%) was potassium chloride (KCl).

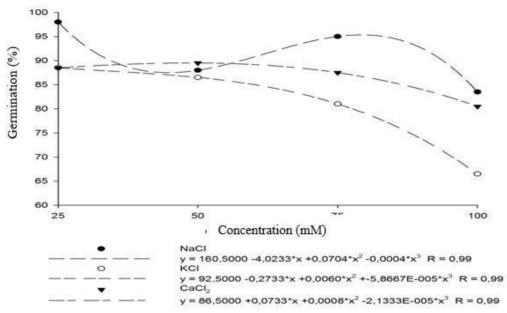


Fig.7: Germination (%) of Acacia mangium Willd seeds. in different concentrations of NaCl, KCl, and CaCl2.

Ferreira et al. (2013) FERREIRA et al. (2013) observed the same trend with the *Cedrela odorata* species, where the NaCl, KCl and CaCl2 concentrations reduced the germination potential of the seeds, the reduction was more drastic in the presence of CaCl2 and KCl, where the most pronounced decrease of germination occurred potentials from 50 mM. Medeiros (2015) MEDEIROS et al. (2015) with Australian scepter seeds submitted to different concentrations of NaCl observed the reduction in the germination potential with the increase of the salt concentration, mainly from 50 mM.

The IVG (Figure 8) presented the same germination

tendency (%) and fit into the cubic equation with R = 0.99. Seed vigor is most affected by the KCl and CaCl2 salts, especially from 75 mM.

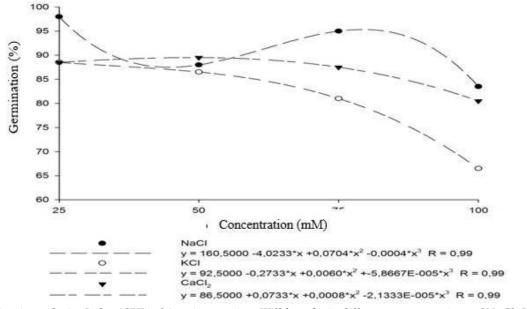


Fig.8: Germination velocity Index (GVI) of Acacia mangium Willd seeds. in different concentrations of NaCl, KCl, and CaCl₂.

Fonseca & Perez (1999) FONSECA and PEREZ (1999) indicated that salinity affects the IVG of seeds of Anadenanthera pavonina L. and Ferreira et al. (2013) FER-REIRA et al. (2013) that the seeds of Cedrela odorata germinated progressively slower from the concentration of 25 mM for the three salts.

Effects on shoot length and Acacia mangium root radius caused by the salts are observed in Figure 9. In the aerial

part, size reduction is observed from the 75 mM concentration in all the salts, however, the salt that most affected the initial growth of the part acacia was CaCl2. The length of the radical was affected from 50 mM in all salts, with the exception of NaCl at the concentration of 75 mM. KCl and CaCl 2 more affect the initial growth of acacia roots than NaCl.

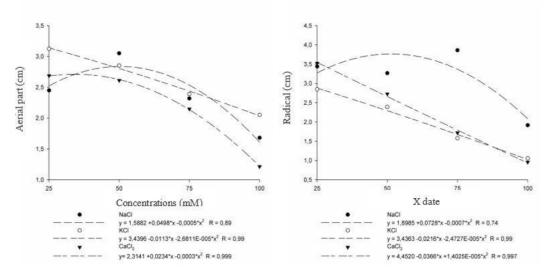


Fig.9: Length of aerial part and radical of seeds of Acacia mangium Willd. in different concentrations of NaCl, KCl, and CaCl₂.

Lima & Torres (2009) LIMA and TORRES (2009) also observed that with the increase of the salt concentration there is a decrease in the initial growth of juazeiro seedlings. Oliveira et al. (2007) 526 (2007) found that the increase of salt concentrations caused reduction of aroeira seedlings (Myracrodruon urundeuva FrAll) and Ribeiro et al. (2008) RIBEIRO et al. (2008), observed that the height of sage saplings (Mimosa caesalpiniaefolia Benth.) Decreased when submitted to salinity.

Saline soils can be found in the field, and the seeds will have to be strong to withstand the adverse conditions found in the environment BERTAGNOLLI et al. (2004), because the seeds are very vulnerable to the effects of salinity, with the increase of saline levels there is initially decreasing the water absorption, thus modifying the imbibition process FERREIRA and REBOUÇAS (1992).

When the concentration of salts in the soil increases, there is a decrease in the osmotic potential causing a decrease in water potential, which can affect the kinetics of water absorption by the seeds (osmotic effect) and raise the concentration of ions in the embryo (toxic effect) (1994).

According to Larcher (2004), LARCHER and Rima (2004), initial seedling development processes are sensitive to the effect of the salts so that growth rate and biomass production are good criteria for assessing the degree of stress and the plant's ability to overcome the saline stress.

IV. CONCLUSIONS

The factor that most affected the germination process of *Acacia mangium* Willd seeds. was the salinity, with the increase of the salts concentration, there was a reduction of the germination, germinating less than 70% in 100 mM CaCl2, which together with the KCl were the salts that most affected the germination, IVG, and size.

Regarding the amount of water in the substrate, germination presented above 94% in all treatments, but better results were found in the amount of 2.5 times the substrate weight, being that below that amount and above there is lower germination. In the aerial part and radical, there is decrease with the increase of the amount of water.

Acacia seeds germinate above 80% in all pHs, but the best results are pH 3.

The results showed that the seeds of Acacia mangium Willd. they do not germinate well with high concentrations of salts and nor with excess humidity, causing physiological changes in the seeds, and that they can develop well in extremely acidic soils.

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