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Hydroponics growth of *Eucalyptus saligna* Sm. on salt-stress mediated by sodium chloride

Crescimento hidropônico de *Eucalyptus saligna* Sm. sob estresse salino mediado por cloreto de sódio

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ABSTRACT

The aim of this work was to evaluate the growth of two clones of Eucalyptus saligna on salt-stress mediated by NaCl in hydroponics. Micropropagated plants of the clones p_0 and p_1 were acclimatizated and cultivated in hydroponics at 0 and 300 mM NaCl levels. The total length, volume, number, fresh mass and dry mass of the roots, the height, fresh and dry mass of the aerial part and the fresh and dry mass of the complete plant were evaluated to the 14 days of hydroponic culture with NaCl. There were significant differences among the clones. The clone p_0 was superior to the clone p_1 in relation to volume of the roots, root number, root fresh mass and total fresh mass of the root. Regards the effect of the salinity on the plants, significant reduction was observed in the height of the aerial part, fresh mass of the aerial part and the total fresh mass. Even so, the interaction between the clones and the concentrations of NaCl was significant for the total fresh mass. In the period of 14 days of hydroponic culture on 300 mM NaCl was possible to discriminate these two clones in relation to the tolerance and susceptibility to the salt stress. The clone p_0 presented higher growth and larger tolerance to the salinity than clone p_1.

Key-words: *NaCl, abiotic stress, eucalypt, salinity; stress tolerance*

INTRODUCTION

Species of the genus *Eucalyptus* present high growth rates (approximately 100 $m^3.ha^{-1}.year^{-1}$) and short rotations (5-7 years for cellulose and paper production) that represents great reasons for the large use of *Eucalyptus* for commercial reforestation in many parts of the world (Ho et al., 1998). Nevertheless, the increase of the soil salinisation is a consequence of the irrigated cultivations, which results in salt accumulations in harmful levels for the plants. This result in a reduced productivity and some of these soils are unable for the use of agricultural practices.

The inhibition of the growth and yield is due the reduction in the osmotic potential caused by the excess of salts and/or to their toxicant effect. In most of the saline soils, sodium is the main adsorbed cation, because in most cases it is in greater amount in relation to the other cations (Marschner, 1995). NaCl reduces the efficiency of use of the nutrients, although its translocation is not affected (Silva et al., 2000; Rego et al., 2011).

To overcome the problem of soil salinisation it is recommended the culture of salt stress tolerant plants. Salt tolerance is the ability of crops to produce an economical yield under adverse soil conditions in the presence of excessive salts in the root zone (Bhutta et al., 2004). However, into a segregant population (i.e. formed by the progenies

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of a crossing, individuals genotypically different from each other), usually some individuals are more tolerant, while others are more susceptible.

The hydroponics represents an excellent tool to evaluate different abiotic stresses, such as salinity and toxicity of heavy metals, besides also it allows to evaluate the effects of the deficiency of certain nutrients for the simple reason to allow a great control of the nutritional conditions. Countless studies have used hydroponics for these purposes, however we can mention some, such as: boron deficiency in pineapple (Siebeneichler et al., 2008), zinc toxicity in *Eucalyptus maculata* and *Eucalyptus urophylla* (Soares et al., 2001) and saline stress in banana tree (Neves et al., 2002).

The aim of this research was to evaluate the growth of two *Eucalyptus saligna* clones on salt-stress mediated NaCl in hydroponics.

MATERIAL E METHODS

Plant material

The clones used in this research were obtained through the *in vitro* germination of seeds. These seeds were supplied for EMBRAPA - Forests, these seeds were a mixture of the following progenies: BR00-519 (27%), BR00-523 (13%), BR01-197 (12%), BR00-534 (12%), BR01-201 (12%), BR00-530 (12%) and BR00-539 (12%). *In vitro* germination was realized followed the methodology described by Lopes da Silva et al. (2010). The clone p0 presented relative tolerance to the salinity in *in vitro* tests and the clone p1 presented susceptibility to the *in vitro* salinity. The letter p refers to our internal control of establishment of clones. The number 0 represents the absence of susceptibility and 1 the presence of susceptibility to the salinity. These clones were multiplicated *in vitro* by indirect organogenesis (Dibax, 2007).

Micropropagated plantlets of *Eucalyptus saligna* 3.3 cm in height (aerial part) were acclimatizated in a glasshouse. Plantlets were transferred to polypropylene dibble tubes (40 mm diameter and 125 mm in height and filled with 50 cm^3 of a commercial substrate named Plantmax® HT) and stayed for 20 days at intermittent nebulization, after this period they were removed from nebulization and daily irrigated during 80 days until the hydroponics experiment.

Salt treatments in hydroponics

Plantlets were removed from dibble tubes; their roots were washed with water tap (faucet) and the plantlets (4.5 cm in height of the aerial part) were cultured in an alveolated tray with thick sand as a substrate (≥ 1 mm). This tray stayed on a nutritive solution composed with half strength MS medium salts, except for Fe-EDTA was used a quarter strength (Murashige and Skoog, 1962). Myo-inositol and vitamins were not added. This solution stayed inside a basin and pH was adjusted to 5.8 each three days, and solution level was adjusted to one liter with distilled water. For each plant was used 10 mL solution. Hydroponic solution was oxygenated with aid the air compressor (1.5 $L.min^{-1}$), which resulted in 202 $L.day^{-1}$. Before experiment installation, plantlets stayed in hydroponics (no treatments) during seven days for adaptation. The experiment was placed at a growth room with a temperature at 25±2ºC and 16 h of photoperiod, under light intensity of 30 μM m^{-2} s^{-1} obtained by white fluorescent lamps. Total length (cm), volume (mm^3), number, fresh and dry mass (mg) of the roots; height (cm), fresh and dry mass (mg) of the aerial part and fresh and dry mass of complete plant were evaluated at 14 days of hydroponic culture with NaCl (21 day of hydroponic culture). Root length was measured with aid of the Win Rhizo Mac La1600 equipment made by Instruments Régent. Dry mass was determined after treatment in oven at 80º C for 24 h.

Experimental design was a complete randomized in a factorial arrangement (2x2), with two clones (p_0 and p_1) and two NaCl levels (0 e 300 mM). It was used five replicates with four plants. The data were submitted to the Bartlett's test for homogeneity of variances, followed by ANOVA (analysis of variance) at the level of significance $P \leq 0.05$. Data from counting were transformed to $\sqrt{x+0,5}$ (i.e. to force data to assume a normal distribution, what is necessary to validate the analysis of variance). All the analyses were done following the procedures of the software GENES (Cruz, 2001).

RESULTS AND DISCUSSION

Both the clones suffered severe leaf distortion at fourth day cultured at 300 mM NaCl. Higher levels of salts generate a low water potential in the solution, which promotes great difficult for plant to acquire water and nutrients. Therefore, salt-stress results in a water deficit condition in plant and it seems a drought physiological condition (Mahajan and Tuteja, 2005).

There were significant differences for factor growth of the clones. Clone p_0 was superior for root volume, root number and total fresh mass (Tables 1 and 2). Nevertheless, there were not statistical differences for root length, height of the aerial part, fresh mass of the aerial part and dry mass of roots, aerial part and total in these clones (Tables 1 and 2).

About the effect of the salinity on the plants, significant reduction was observed in the height of the aerial part, fresh mass of the aerial part and total fresh mass (Tables 1 and 2). Similar results were found in banana plant cultivated in hydroponics at 5, 10 and 15 mmol L^{-1} sodium level, which promoted significant reduction in the fresh mass of the aerial part and in the height of the aerial part after almost two months of culture (Neves et al., 2002). However, the other variables did not present statistical differences. Nevertheless, interaction between the clones and the NaCl levels was only significant for the total fresh mass (Table 1). This result suggest the period of 14 days of hydroponic culture of the *Eucalyptus saligna* plants can be used to discriminate tolerant and susceptible clones to the salinity. However, probably larger periods could allow more precise discrimination.

Table 1. Summary of two-way ANOVA (analysis of variance) of effects of salinity and clones on growth of *Eucalyptus saligna*. Root total length (CR cm), root volume (VR cm^3), heigth of the aerial part (A cm), root number (NR), fresh mass of the aerial part (MPA mg), total fresh mass (MFT mg), dry mass of the aerial part (MSP mg), root dry mass (MSR mg) and total dry mass (MST mg) of two clones to the 14 days of hydroponic culture under salt treatment.

		Mean square				
Source of variation	**d.f.**	**CR cm**	**VR cm^3**	**A cm**	**NR**	**MPA mg**
Clones (p_0 and p_1)	1	1988.9 ^{ns}	519493.8 *	0.023 ^{ns}	0.9807 *	2989.01 ^{ns}
NaCl (0 and 300 mM)	1	50.2 ^{ns}	52020.0 ^{ns}	25.205 *	0.0006 ^{ns}	126537.5 *
Clones x NaCl	1	1732.3 ^{ns}	30420.0 ^{ns}	0.002 ^{ns}	0.1090 ^{ns}	318.66 ^{ns}
Residual	16	922.7	56677.3	0.905	0.0946	3201.4
CV(%)		27.1	29.2	16.7	12.3	31.3
		Mean square				
Source of variation	**d.f.**	**MR mg**	**MFT mg**	**MSP mg**	**MSR mg**	**MST mg**
Clones (p_0 and p_1)	1	80978.7 *	115083.4 **	45.69 ^{ns}	106.56 ^{ns}	271.49 ^{ns}
NaCl (0 and 300 mM)	1	4498.7 ^{ns}	83317.9 **	780.98 ^{ns}	12.14 ^{ns}	569.11 ^{ns}
Clones x NaCl	1	27878.6 ^{ns}	83317.9 **	492.73 ^{ns}	23.83 ^{ns}	766.47 ^{ns}
Residual	16	6834.2	16832.5	294.83	84.29	674.08
CV(%)		32.2	25.3	35.2	30.9	33.1

*Significant at the level of 1% of error probability for the test F, ** Significant at the level of 5% of error probability for the test F, ^{ns} Not significant.

Table 2. Characteristics observed at 14 days of hydroponic culture under saline stress of two clones of *Eucalyptus saligna*.

	Root length (cm)			**Root volume (mm^3)**		
NaCl	**Clones**			**Clones**		
(mM)	**P_0**	**P_1**	**Mean**	**P_0**	**P_1**	**Mean**
0	111.5	109.0	110.2	779.3	435	557.1 a[1]
300	132.7	89.2	110.9	759.3	459	659.1 a
Mean	122.1	99.1		769.3 A	447 B	
	Height of the aerial part (cm)			**Root number**		
NaCl	**Clones**			**Clones**		
(mM)	**P_0**	**P_1**	**Mean**	**P_0**	**P_1**	**Mean**
0	6.8	6.7	6.7 a	7.3	4.2	5.7 a
300	4.4	4.5	4.4 b	6.7	5.0	5.8 a
Mean	5.6	5.6		7 A	4.6 B	
	Fresh mass of the aerial part (mg)			**Root fresh mass (mg)**		
NaCl	**Clones**			**Clones**		
(mM)	**P_0**	**P_1**	**Mean**	**P_0**	**P_1**	**Mean**
0	235.4	203.0	219.2 a	283.9	181.3	232.6 a
300	68.3	51.9	60.1 b	288.6	136.7	212.6 a
Mean	151.8	127.4		286.2 A	159.0 B	
	Total fresh mass (mg)			**Dry mass of the aerial part (mg)**		
NaCl	**Clones**			**Clones**		
(mM)	**P_0**	**P_1**	**Mean**	**P_0**	**P_1**	**Mean**
0	469.4Aa[2]	384.3Ba	426.8 a[2]	51.5	58.4	54.9
300	407.0Ab	188.6Bb	297.8 b	48.9	36.0	42.4
Mean	438.2 A[2]	286.4 B		50.2	47.2	
	Root dry mass (mg)			**Total dry mass (mg)**		
NaCl	**Clones**			**Clones**		
(mM)	**P_0**	**P_1**	**Mean**	**P_0**	**P_1**	**Mean**
0	30.0	27.6	28.8	81.0	86.0	83.5
300	33.8	27.0	30.4	82.7	63.0	72.8
Mean	31.9	27.3		81.8	74.5	

[1]Means followed by different capital letters in the lines presents differences and means followed by different lower case letters in the columns presents differences, both at 1% for test F. [2] Means followed by different capital letters in the lines presents differences and means followed by different lower case letters in the columns presents differences, both at 5% for test F. Variables without letters in the columns and lines were not significant.

The variables affected by the salinity (300 mM NaCl) presented a reduction in percentage with relation to the control (0 mM NaCl) of 34.3%, 72.6% and 30.2% for the height of the aerial part, fresh mass of the aerial part and total fresh mass, respectively. The only variable that identified the level of 300 mM NaCl as a lethal dose (DL_{50}) was the fresh mass of the aerial part, which was drastically reduced in a percentage below 50% of the normal parameter of growth. However, the severity of the stress level is relative to the plant age and to the time of exhibition (Hoffman and Rawlins, 1971). Due to the fact of the plants stayed a long time in the dibble tubes before the installation of the experiment, its root system was already very developed, what can have resulted in the low susceptibility to the saline treatments, whereas there was little space in cells for the development of the roots. Therefore it is probable that the aerial part was more susceptible to salinity than the roots.

The tolerance to the saline stress can be due to the control of the acquisition and for the allocation of sodium inside the plant, or for the osmotic

readjustment and other physiologic processes (Cheeseman, 1988). The inhibition of the growth and the plant production is due to the reduction in the osmotic potential caused by the excess of salts and/or to the toxicant effect of the same ones (Silva et al., 2000).

The more important organic solutes accumulated in superior plants under conditions of salt stress are carbohydrates and proline. The proline level is increased when the plant suffers some stress type related to the osmotic adjustment. The proline is an osmoprotector that works as an osmolyte, which acts in the osmotic adjustment of the cell, aiding to maintain the hydric balance of the plant, without there are decreases of the turgor or cellular volume (Taiz and Zeiger, 2004). The largest tolerance the salinity of the Clone p_0 can be due to the larger produced amount of sugar soluble and proline than the clone p_1.

CONCLUSION

The clone p_0 presented higher growth and larger tolerance to the salinity than clone p_1.

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RESUMO

O objetivo desse trabalho foi avaliar o crescimento de dois clones de Eucalyptus saligna sob estresse salino mediado por NaCl em cultivo hidropônico. Plantas dos clones p_0 e p_1 micropropagadas foram aclimatizadas e cultivadas em hidroponia sob 0 e 300 mM de NaCl. Foram avaliados o comprimento total, volume, número, massa fresca e seca das raízes, a altura, massa fresca e seca da parte aérea e a massa fresca e seca da planta completa aos 14 dias de cultivo sob NaCl. Quanto ao crescimento dos clones houve diferenças significativas entre os mesmos. O clone p_0 foi superior ao clone p_1 com relação às variáveis, volume das raízes, número de raízes, massa fresca das raízes e massa fresca total. Quanto ao efeito da salinidade sobre as plantas, foi observada significativa redução na altura da parte aérea, massa fresca da parte aérea e a massa fresca total. Porém, a interação entre os clones e as concentrações de NaCl foi significativa para a massa fresca total. No período de 14 dias de cultivo hidropônico sob 300 mM de NaCl foi possível discriminar esses dois clones com relação à tolerância e à suscetibilidade ao estresse salino. O clone p_0 apresentou maior crescimento e maior tolerância à salinidade do que o clone p_1.

Palavras-chave: *NaCl, estresse abiótico, eucalipto, salinidade, tolerância ao estresse*

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