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# Effect of NaCl on willow hidroponyc experiment

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**Abstract** The aim of this study is to present the influence of NaCl on willow cuttings growth in a laboratory experiment. Two factorial hydroponic experiment on cuttings of (i) seven willows (two Romanian hybrids from National Institute for Research and Development in Forestry "Marin Drăcea":RO892, RO1082; three Romanian willow genotypes from CHPs Govora: Cozia, Fragisal, Robisal; two Swedish willow hybrids:Olof, Tordis), (ii) in three salinities (E1-50nM (5,85g/l); E2 - 100nM (8,80g/l); E3 – 200nM (11,70g/l)) plus a control were carried out. Under saline stress, most of the shoots wilted but dry biomass production and proline content were calculated in order to highlight the response of wilow clones to different salinity stress. Significant differences were determined between clones, the less productive one in term of shoot biomass being Cozia. No pattern was established for saline concentration and proline content in root tissues.

**Key words**

willow, hydroponic experiment, NaCl, dry biomass

Salinity is an abiotic stress factor that limits plant development and drastically reduces the yield if the salt concentrations are high (Allakhverdiev et al., 2000, Parida et Das, 2005, Shannon 1998). Research made in agricultural crops showed that salt tolerance differs according with species and also among cultivars within the same species (Katerji et al. 1992). 0nly few studies were made in order to test the salt tolerance of willows. Usually, these studies use growth and yield measurements to identify salt tolerance levels. Research highlight that some willows are drought tolerant, (Mang and Reher, 1992; Gray and Sotir, 1996; Hightshoe, 1998) and the salt tolernace range from sensitive to moderately tolerant. (Mirck and Zalesny, 2015). The experiment made in Canda showed that some varieties were able to tolerate moderate or even severe saline concentration (Hang et al. 2011). It is well known the willows wide genetic variation and also the differences between cultivars not only species in agricultural crops. In this context, the discover of tolerant willow crops is a challange (Mirck and Zalesny, 2015).

In Romania, about 4,4 million ha of land are affected or potentially affected by salinity (Tesileanu & Fedorca, 2015).

Willow short rotation coppice is a potential crop to better use of saline marginal land (Hang et al. 2011) so improve the knowledge of willow short rotation coppice of salt tolerance is important (Mirck and Zalesny, 2015)

The aim of this study is to present the influence of NaCl on willow cuttings growth in a laboratory experiment.

## Materials and Methods

A two factorial hydroponic experiment on cuttings : (i) species, (ii) NaCl solution was carried out. Seven willow clones: two Romanian hybrids from National Institute for Research and Development in Forestry "Marin Drăcea" (RO892, RO1082), three Romanian willow genotypes from The Fruit Growing Research and Development Unit of Vâlcea (SCDP Vâlcea) and CHPs Govora (Cozia, Fragisal, Robisal) and two Swedish willow hybrids (Olof, Tordis) from a Hungarian Nursery were used. Three different saline solution (E1-50nM); E2 - 100nM); E3 – 200nM ) plus a control (C) was the other experiment factor. The cuttings 10 cm length an average of 2-4 buds/cutting) were put in 160ml non-transparent plastic pots filled with tap water. After two weeks the tapwater was changed with Hoagland solution with different saline concentration, that was maintained for another two weeks. Measurements and observations were made on a total number of 252 cuttings at the beginning of the experiment, and then before and after saline treatment: (i) diameter at the middle of the cutting, (ii) weight of the cutting, (iii) the length of the higher shoot, (iv) dry biomass of shoots (v) dry biomass of roots. Dry biomass was established by weighed after drying at $105^0$C until constant weight. In order to account the differences dry biomass was calculated as the ratio of the dry mass shoot: cutting initial mass, dry mass root: cutting initial mass and multiplied by hundred (Heike et al., 2014).

Vitality was determined at the end of the experiment according to five predefined vitality classes (5: high, leaves green; 4: medium, leaves up to 50% necrotic; 3: low, leaves more than 50% necrotic; 2: leaves totally necrotic; 1: leaves and shoots stem totally necrotic) (Heike et al., 2014 modified).

The determination of proline content in root was made by calorimetric method measuring the absorbance at 520 nm. The fresh tissue was homogenized in 3% sulfosalicylic acid and the color reaction in ninhydrin acid was established. According to Bates (1973), L-proline standard concentration curve was used.

The mean and standard deviation of the recorded data were calculated using STATISTICA 10 software. After applied the treatment (saline solution) most of the shoots wilted and some of them died so the statistical analysis was developed for measurements made after two weeks in order to highlight the differences between clones. Results were displayed in table and graphs. In order to account the differences in initial weight of cuttings, the percent of shoots dry biomass of the cutting initial mass of cutting, respectively the percent of roots dry biomass of the cutting initial mass of cutting were calculated

## Results and Discussions

The cuttings used in this hydroponic experiment are about 10cm length but the diameters are different depending on clones (Table 1). The shoots length measured after two weeks at the beginning of the experiment were also different, related probably with clones and initial diameters (Table 2).

After two weeks of salinity treatments on cuttings, the vitality of the shoots, as well as of the roots generally decreased with increasing salinity level (Table 3, Fig.1). Similar results were obtained by Heike et al. (2014) in an experiment on *S.alba* and *S. viminalis*.

ANOVA test revealed that both the genotype and the salinity treatment had a significant effect on growing and rhisogenesis process, as well as on dry mass of the shoots and roots (Table 4).

The response of willow clones to different salinity stress are shown in graphs below (Fig. 2 and 3).

Table 1

**Statistical parameters for cutting diameter**

| clone | No of cuttings | Cutting diameter (mm) | |
|---|---|---|---|
| | | Mean | St.dev |
| RO892 | 36 | 10,19 | 1,39 |
| RO1082 | 36 | 9,02 | 1,67 |
| Cozia_1 | 36 | 9,34 | 1,35 |
| Fragisal | 36 | 8,87 | 1,016 |
| Robisal | 36 | 10,46 | 1,43 |
| Olof | 36 | 8,95 | 1,01 |
| Tordis | 36 | 12,18 | 1,05 |

Table 2

**Statistical parameters for shoots length before NaCl treatment**

| clone | Exp. variant | No of cuttings | Shoots length (mm) | |
|---|---|---|---|---|
| | | | Mean | St.dev |
| RO892 | E1 | 9 | 15,31 | 2,68 |
| | E2 | 9 | 14,19 | 3,70 |
| | E3 | 9 | 12,67 | 2,69 |
| | control | 9 | 12,33 | 2,24 |
| RO1082 | E1 | 9 | 10,11 | 2,20 |
| | E2 | 9 | 10,00 | 3,84 |
| | E3 | 9 | 9,11 | 4,11 |
| | control | 9 | 10,33 | 1,58 |
| Cozia_1 | E1 | 9 | 2,83 | 2,46 |
| | E2 | 9 | 4,94 | 3,84 |
| | E3 | 9 | 5,70 | 2,28 |
| | control | 9 | 5,71 | 2,25 |
| Fragisal | E1 | 9 | 8,89 | 2,37 |
| | E2 | 9 | 7,50 | 2,88 |
| | E3 | 9 | 8,18 | 1,70 |
| | control | 9 | 6,73 | 2.00 |
| Robisal | E1 | 9 | 9,54 | 3,13 |
| | E2 | 9 | 9,41 | 2,84 |
| | E3 | 9 | 10,34 | 3,02 |
| | control | 9 | 9,06 | 3,05 |
| Olof | E1 | 9 | 8,07 | 1,27 |
| | E2 | 9 | 9,77 | 2,77 |
| | E3 | 9 | 10,60 | 3,12 |
| | control | 9 | 11,84 | 2,15 |
| Tordis | E1 | 9 | 10,31 | 4,14 |
| | E2 | 9 | 11,93 | 3,43 |
| | E3 | 9 | 11,01 | 2,74 |
| | control | 9 | 10,14 | 1,92 |

Table 3

**Shoots vitality after two weeks of salinity treatment**

| clone | Control | Salinity treatment | | |
|---|---|---|---|---|
| | | E1 | E2 | E3 |
| RO892 | 5 | 4 | 2 | 2 |
| RO1082 | 5 | 4 | 1 | 1 |
| Cozia_1 | 5 | 3 | 1 | 1 |
| Fragisal | 5 | 3 | 2 | 2 |
| Robisal | 5 | 3 | 2 | 1 |
| Olof | 5 | 4 | 2 | 2 |
| Tordis | 5 | 3 | 2 | 2 |

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Fig.1.The vitality of the shoots according to clone and experimental variants

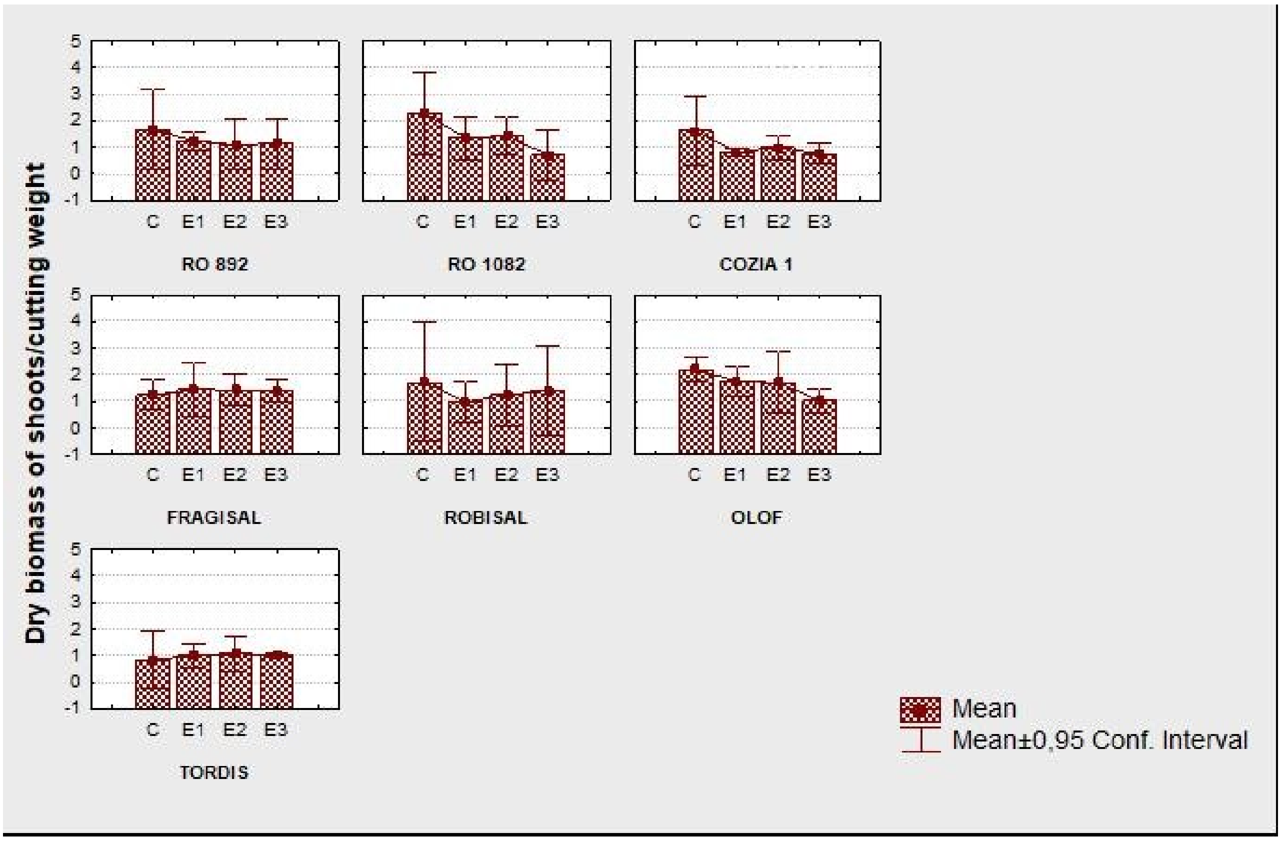


Fig. 2 . Dry biomass of shoots/cutting weight, according to clone and experimental variants

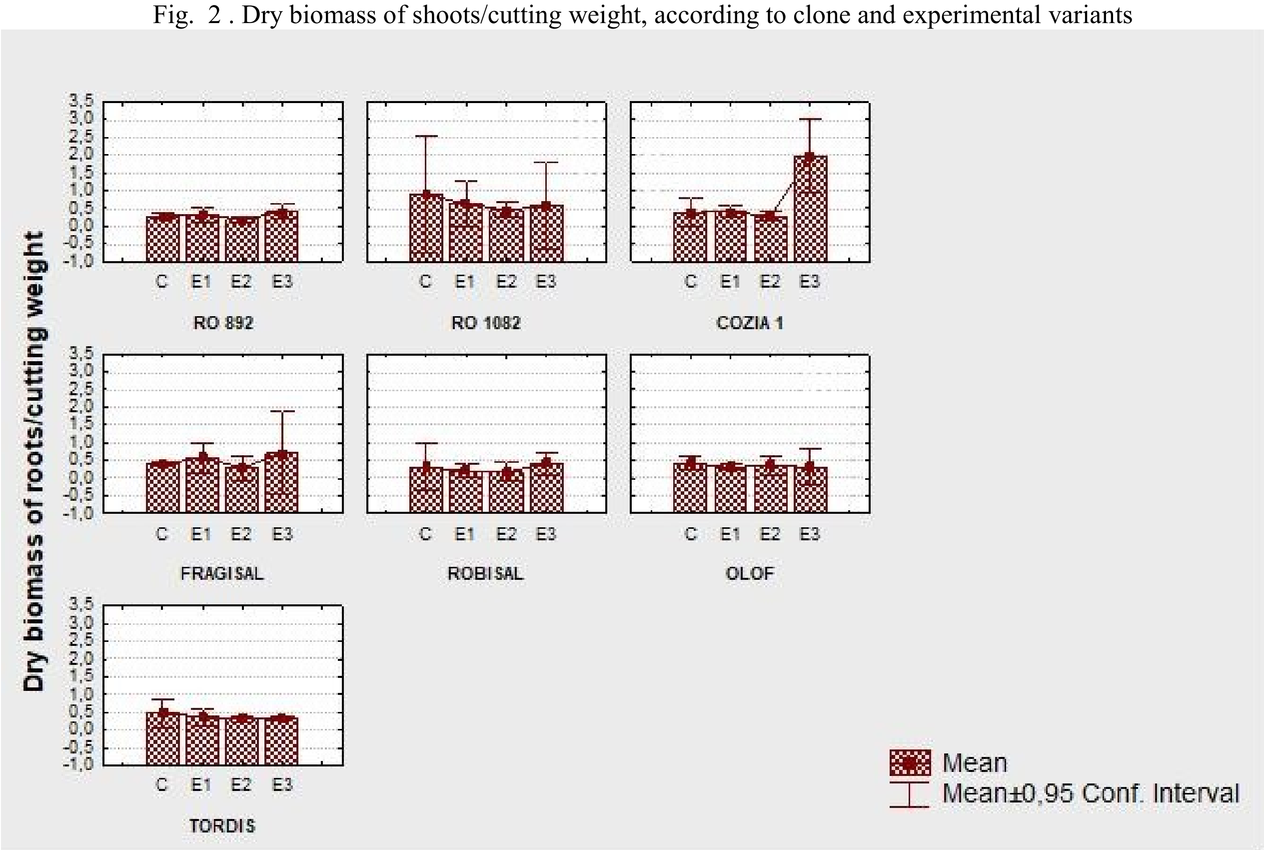


Fig. 3 . Dry biomass of roots/cutting weight, according to clone and experimental variant

Table 4

**The effect of genotype and salinity treatment on analyzed characters (ANOVA test)**

| Analysis of Variance ( Marked effects are significant at p <0.05000) 1 = clone 2 = salinity treatment | | | |
|---|---|---|---|
| Character | Factor | F | p |
| % shoots growing | 1 | **21.575700*** ** | **0.000000** |
| | 2 | **3.394656*** | **0.018562** |
| | 1x2 | **6.036112*** ** | **0.000000** |
| % new roots no | 1 | **7.394340*** ** | **0.000000** |
| | 2 | 1.354907 | 0.257191 |
| | 1x2 | **2.506529*** ** | **0.000130** |
| Dry biomass of the shoots | 1 | **2.959241*** | **0.011847** |
| | 2 | **5.946747** ** | **0.001030** |
| | 1x2 | **3.171313*** ** | **0.000133** |
| Dry biomass of the roots | 1 | **3.226377** ** | **0.007013** |
| | 2 | **4.004698*** | **0.010390** |
| | 1x2 | **6.676229*** ** | **0.000000** |

The reaction of the neoformed shoots and roots is dependent mainly on genotype and less on salinity treatment. Generally, the growth rithm (evaluated as the percent of new growth) is similarly in E1 with Control and decreased than with increasing salinity (Table 5). An exception is RO 892, where even the lowest salt concentration inhibited the growing process. The same inhibition of the shoots growing process was observed by Heike et al. (2014). Regarding the rhisogenesis process (evaluated as the percent of newly formed roots) the clones can be split into three categories: a. the process is enhanced in RO 1082, Olof and Tordis; b. the process is inhibited in RO 892, Cozia, Fragisal; c. no effect of the salinity treatment in Robisal (Table 5).

A critical role in protecting plants from stress, particularly under saline conditions might be played by proline. Proline was studied in numerous works dealing with plant selection against abiotic stresses such as drought and salinity (Marin et al. 2010)

For genus *Prunus*, like a response to increasing NaCl concentrations, the proline concentrations increased (Marin et al. 2010), but a different proline accumulation pattern was found for willow experiment. The proline concentration varies with both the genotype and experimental variants (saline solution). Only for Cozia and Olof genotypes, the proline concentration increased as salt concentration increased to 50nM. All other genotypes react differently to different salt concentration (Table 6).

Table 5

**The effect of genotype and salinity treatment on shoots growth and rhisogenesis**

| Clone | Var. | % shoots growing | % new roots no. |
|---|---|---|---|
| RO 892 | **C** | **19.77 a** | **77.94 a** |
| | E1 | 6.95 **b** | 52.41 **b** |
| | E2 | 7.29 **b** | 55.25 **b** |
| | E3 | 5.05 **b** | 56.30 **b** |
| RO 1082 | **C** | **12.88 a** | **36.35 c** |
| | E1 | 13.69 **a** | 76.67 **a** |
| | E2 | 4.26 **b** | 51.72 **b** |
| | E3 | 6.83 **b** | 51.04 **b** |
| Cozia 1 | **C** | **103.29** | **63.94 a** |
| | E1 | 118.81 **a** | 71.33 **a** |
| | E2 | 74.53 **b** | 47.11 **b** |
| | E3 | 52.62 **b** | 28.90 **c** |
| Fragisal | **C** | **22.02 a** | **94.47 a** |
| | E1 | 5.14 **b** | 70.31 **b** |
| | E2 | 18.91 **a** | 93.47 **a** |
| | E3 | 6.08 **b** | 56.90 **b** |
| Robisal | **C** | **20.30 a** | **29.74 a** |
| | E1 | 16.01 **a** | 34.22 **a** |
| | E2 | 7.33 **b** | 24.60 **a** |
| | E3 | 0.58 **b** | 29.71 **a** |
| Olof | **C** | **26.01 a** | **16.33 b** |
| | E1 | 17.67 **a** | 28.04 **a** |
| | E2 | 16.15 **a** | 39.51 **a** |
| | E3 | 4.85 **b** | 30.02 **a** |
| Tordis | **C** | **45.45 a** | **21.63 b** |
| | E1 | 45.05 **a** | 53.05 **a** |
| | E2 | 23.21 **b** | 30.92 **b** |
| | E3 | 15.26 **b** | 22.23 **b** |

The letters indicate the significance of the difference between the different experimental variant, for each clone, according to DUNCAN test. **a** is considered the highest value,

Table 6

**The proline content in root (µg /1g fw)**

| clone | Experimental variant | | |
|---|---|---|---|
| | Control | E1 | E2 |
| RO892 | 40.09 | 13.02 | 21.57 |
| RO1082 | 27.12 | 20.76 | 5.89 |
| Cozia_1 | 58.88 | 74.68 | 30.06 |
| Fragisal | 27.19 | 17.44 | 47.04 |
| Robisal | 41.85 | 76.16 | 56.48 |
| Olof | 34.53 | 15.24 | N/A |
| Tordis | 39.08 | 10.32 | 68.03 |

## Conclusions

Genotype is the determining factor in tolerance to salinity. Under saline stress, most of the shoots wilted and some of them died. Biomass production and proline content were calculated in order to highlight the response of willow clone to different salinity stress.

Significant differences were determined between clone, the most productive in term of shoot biomass being RO 1082 and OLOF and the less productive one Cozia. No pattern was established for saline concentrations and proline content in root tissues.

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