JOURNAL of Horticulture, Forestry and Biotechnology
Volume 30(1), 2026, 22-30
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

Salt tolerance of Giant reed (*Arundo donax* L.) ecotypes in callus cultures in vitro

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Manuscript received: 15 November 2025; revised: 1 December 2025; accepted: 04 December 2025

Abstract
Nowadays, soil salinity is a big challenge for agronomists and plant growers due to irrigation in arid lands where the respiration rate is very high and in coastal areas saline water floods the soil. Salinity is caused by the accumulation of soluble salts in the root zone. Excess salts reduce the productivity of plants like growing potential, vigor and seed germination, due to the altered water and mineral uptake. Among salts the amount of sodium and chloride ions is very critical because most of the plants are sensitive to them. *Arundo donax* L., commonly known as giant reed, has a wide habitat range thus it has wide tolerance to soil conditions. Hence, it may has high resistance to salinity and sodicity, too. It is a typical plant of freshwater ecosystems. Although *A. donax* is widespread all over the world, it has only slight genetic differences between ecotypes due to its vegetative propagation strategy. *A. donax* can be propagated by *in vitro* tissue culture technique. *In vitro* cell culture is suitable for large scale selection of cells where cells are exposed to stressors such as high salinity or sodium. This form of selection can reduce the time and space requirements, reducing the need for conventional selection in open filed experiments. In our experiment 12 ecotypes of *A. donax* were tested in *in vitro* callus cultures. Callus cultures were exposed to salt stress by adding five different concentrations of sodium chloride (NaCl) to the culture medium. Growth parameters of callus cultures were measured by the fresh and dry matter production, under constant laboratory conditions. The fresh weight of calli decreased while their dry weight increased with increasing NaCl level in culture medium. According to our experiments, differences in NaCl tolerance were registered between the different ecotypes.

Keywords: tissue culture, salt stress, NaCl, callus growth, fresh weight, dry matter content

Introduction

The high soil salinity is a serious and increasing problem all over the world. It is caused by partly the high amount of water soluble salts present in the soil, partly the high evaporation rates in arid and semi arid regions, partly the irrigation water with moderate salin content. It is also caused by the flooding of sea water in costal regions. Soil salinity can be measured by the electric conductivity of the soil solution. Both the higher amount of water soluble salts in the soil that plants can tolerate and the reduced amount of water in the soil cause salt stress for plants. Plants species has different optimum for different salts hence they have different sensitivity against them. Water-soluble salts are present in the solution as their ions such as K^+, Na^+, Ca^{2+}, Mg^{2+} (anions), and Cl^-, $HCO_3^-SO_4^{2-}$, NO_3^-, PO_4^{2-} (cations). Salt stress mainly means the elevated amount of Na^+ and Cl^- in the soil. However, salt stress is also caused by the higher amount of other ions, such as K^+, Mg^{2+}, Ca^{2+}, or SO_4^{2-} ions, present in the soil. Excess amount of certain ions can alter the uptake of nutritional minerals from the soil [1]. Moreover sodium ion has toxicity to plant cells in higher amounts. It can disrupt the photosyntetic apparatus, inhibit carbohydrate production, cause water and osmotic stress and early leaf senescence. Therefore, the biomass production of the cells and the yield of the crop will decrease [2]. This will cause financial losses in the agriculture. The development of salt tolerant crops can mitigate this problem. Conventional breeding needs several years or decades to delevolope salt tolerant cultivars [3-4]. Salt tolerant plant can tolerate the higher osmotic pressure, or can regulate better the level of Na+ and Cl- in the citosol.

It can inhibit the access of these ions into the cells of assimilating tissue or compartmentalize them in vaquol [5]

Salt stress induces typical physiological changes in plants. Stressed plant cells accumulate water soluble sugars and organic acids caused by osmotic imbalance. Salt stress inhibits the production of starch from sugar. Typical changes happen in protein and free aminacid content. They usually accumulate prolin, glicin or betain (a protein of sugar beet). The level of prolin is a good indicator of the degree of salt stress. Salt stress usually increases the level of carotenoids, ascorbic acid, glutathion and anthocianin, but lower the amount of chlorofill-a and -b. Salt stress - similar to other abiotic stresses - increase their rate of free radicals, the reactive oxigen species (ROS). ROS activate the ROS scavenging system like catalase (CAT), superoxid dismutase (SOD) and ascorbat peroxidase (APX). Dry matter content usually decreases in stressed plants [6, 7]. However it was found that in tissue culture conditions the amount of dry matter content usually increases under lower stress conditions or in case of salt tolerant plant material (see in Table 1).

The salin conditions and the adaptation of the plants to these conditions exist well before the domestication of crops [4]. Thus potential for salt tolerance also exists within the genome of non-halophytes as shown by plant studies. Warne and Hickok [8] made examinations on haploid fern gemtophytes for salt tolerance. They could select salt tolerant lines. They results verified the function of a single gene in salt tolerance. In crop plants it seems likely that differences in salt tolerance exist in the cultivars of outbreeding species or in the segregating population of inbred lines. However these differences are caused by genes with additive effects. The tolarance of plants against saline conditions is rarely caused by mutations in the genomes. Most frequently it is caused by the changes in the activity of genes related to salt tolerance. Epigenetic changes obscure the real mutants with true tolerance. The variations by genetic and epigenetic changes normally exist in plant lives [9].

In tissue culture systems, small plant parts, or the mass of individual cells can be cultured in a relatively small place under controlled conditions. At present, a wide range of species can be propagated by tissue culture. The instability of *in vitro* cultures may causes genetic and epigenetic variations, the so called somaclonal variability (according to Ferreira et al.) [10]. It causes difficulties, when the genetic fidelity is the goal of the culturing process (like as conservation of threatened species), but it is a useful tool for the production of new varieties with improved tolerance. Somaclonal variation among the selection of new varieties is mainly used for ornamentals and crop plants, especially sugarcane, rice, banana, potato and wheat. Somaclonal variation can be induced by the application of plant growth regulators (PGRs) such as benzylaminopurine (BA) or 2,4 dichlorophenoxyacetic acid (2,4D) and some other chemicals. [10]. For the selection of salt tolerant plants *in vitro*, researchers mainly use NaCl as a selective material, applying it in the tissue culture medium. KCL, Na_2SO_4, $MgSO_4$ and K_2SO_4 can be used as selective material as well. Salt resistent plants have a cross tolerance against different salts. It means that NaCl tolerant plants may tolerate KCl, or Na_2SO_4, too [7]. In vitro salt stress experiments were carried out on numerous species in numerous researches, like Flax (Linum ultissimum) [11], Wheat (Triticum eastivum), Kirik variety [12], Mentha (Mentha viridis), wild type [13], Rosewood (Dalbergia sissoo) [14], Limonphila aromatica Lamk. Merr. and Bacopa monnieri L. wettst [15], Rice (Oryza sativa L.) MARDI Siraj 297 variety [16], Onion (Allium cepa L.) Faridpuri, Taherpuri, Pusa varity [17], tomato (Solanum lycopersicum L.) BD-7755, BD-7757, BD-9008, BD-9011 and BD-10122, BD-10123 variety [18], Date palm (Phoenix dactylifera L.) Khalas cultivar [19], Spinach (Spinachia oleracea L.) [20], eggplant (Solanum mmelongena) [21], Thevetia peruviana Schum. [22], Princess tree (Paulownia tomentosa) [23], onion (Allium cepa L.) Giza 6 cultuvar [24], Pistachio UCB-1 hybrid (Pistacia atlantica × P. integerrim) [25], Sugarbeet (Beta vulgaris), Prima Hill variety, Tobacco (Nicotiana tabacum) Wisconsin 38 variety, Chinese cabbage (Brassica campestris ssp. pekinensis) Kimjung variety and Canola (Brassica napus) Westar variety [26], White poplar (Populus alba L.) [27].

The aim of our recent work to determine the differences in salt tolerance capability of our *in vitro* cultured ecotypes and to determine the range of salt percentage which can be applicable in giant reed callus cultures for further expreiments.

Materials and methods

Plant material: Embryogenic callus cultures of 12 Giant reed ecotypes were used. Callus cultures originated from different climatic regions and soil conditions. Embryogenic calli were induced from either immature inflorescence or nodal tissue. Complete method is published in the patent of University of South Carolina [28]

Culture medium: The callus maintaining culture medium was enriched with five different concentration of sodium chloride (0.1, 0.3, 0.5, 0.7 and 0.9%, which correspond to 1, 2, 5, 7 and 9 g/l, that is 17, 51, 85, 120 and 154 mM, respectively). The pH of the medium was set to 5.7-5.8 prior to autoclaving at 120°C temperature, for 20 minutes. Cultures were caped with one hole cap (5mm of diameter), and sponge were across the hole to avoid contamination of cultures. The composition of the callus maintaining culture medium can be found in the patent of University of South Carolina [28]

Experimental design: 50g (49.5-50.5g) of sterilized culture medium was poured into sterilized jar, with the volume of 390ml, measured on scale under sterile conditions. 9g (8.9-9g) of callus tissue was added to the measured culture medium. 12 ecotypes of giant reed were tested (labeled ES04, BCF24, SÓS24, SÓS1, SÓS2, V2, GT, TCS, A1708, 08, 20SZ, BL). Every ecotypes was collected from different fields. Six experimental lines were made with every ecotypes (cultures with 5 different concentration of NaCl and controll culture). One treatment consisted of 6 cultures. Cultures were maitained in a culturing room at 23°C temperature and 16/8 photoperiod provided via a 1:1 mix of daylight and warm-white, fluorescent lamps in a vertical position, resulting in a light intensity of 25-30 $\mu mol\ s^{-1}\ m^{-2}$. Culturing period lasted 4 weeks.

Data collection and statistical analysis: Data were collected at the end of the subculture, after 4 weeks. The fresh weight of callus tissue (g) and the the dry matter content of callus tissue - after 1 day drying on 60°C temperature (percentage of dry/fresh weight of calli) - per jar were measured. Measurements were made from 6 jars in each treatment. SPSS for Windows software (SPSS® version 21.0) was used for statistical analysis. One-way ANOVA followed by Duncan's test at $p < 0.05$ was applied to analyze the data of both experiments to compare the effect of different treatments.

Results and Discussions

Callus cultures of every ecotypes showed decreased growing potential (based on the fresh weight of callus tissue) under sodium chloride stress, while the dry matter content of callus tended to increase with the increasing concetration of NaCl in the culture medium (Table 1 – 12). Differences were observed in growing potential and dry matter content among ecotypes both under normal conditions i.e. without salt stress (Table 13) and under salt stress too (Table 14 – 18). Based on the fresh weight on control medium, the best growing potential was measured in case of V2 ecotype (31.0±0.64g, Table 13), while the biggest amount of dry matter content was in case of 08 ecotype (9.81±0.22%, Table 13). The growing potential changed above 0.3% NaCl content of the culture medium (Table 16). The highest fresh weight was given by the BL ecotype, while the dry matter content remained the highest in the case 08 ecotype until 0.9% salt content. At 0.9% NaCl content of the culture medium, dry matter content was the highest in case of ES04 ecotype (11.6±0.28%, Table 19). The fresh weight of the callus culture decreased in the highest degree in case of V2 ecotype (11.4g, Table 3., Table 13), while the smallest loss was in case 08 ecotype (1.7g, Table 12, Table 13). The growth of dry matter content was the highest in case of ES04 ecotype (4.5g, Table 1, Table 13), while the lowest was in case of A1708 ecotype (1g, Table 10, Table 13).

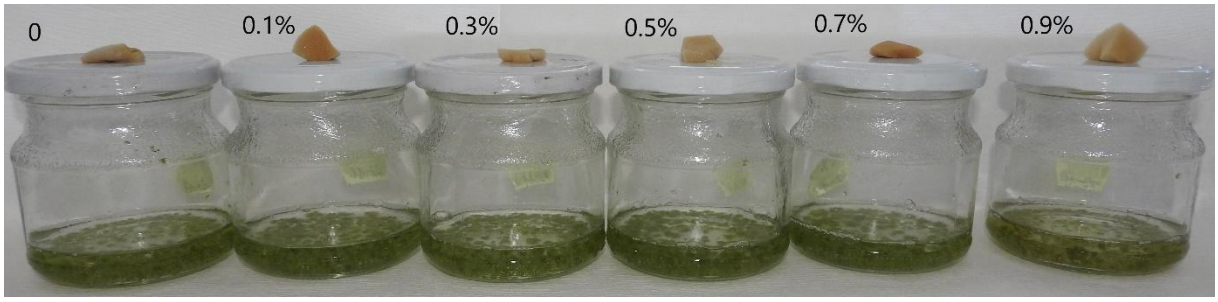

Figure 1. Salt treatment of BL ecotype (NaCl concentration is indicated on the picture)

Table 1. Mean values of measured parameters with standard errors in salt tolerance experiment of ES04 ecotype. Mean values are followed by different letters that indicate significantly ($p<0.05$) different values between treatments according to ANOVA and Duncan tests.

Concentration, ES04	fresh weight (g/culture)	dry matter content (%/culture)
Control	23.2±0.50a	7.1±0.15d
0.1%	22.9±0.76a	7.7±0.14c,d
0.3%	22.8±0.54a	7.7±0.11c,d

0.5%	21.1±0.75b	8.1±0.33c
0.7%	17.9±0.45c	9.7±0.36b
0.9%	15.2±0.12d	11.6±0.28a

Table 2. Mean values of measured parameters with standard errors in salt tolerance experiment of V2 ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests.

Concentration, V2	fresh weight (g/culture)	dry matter content (%/culture)
Control	31.0±0.64a	5.5±0.06e
0.1%	30.7±0.85a	5.7±0.05e
0.3%	26.5±1.04b	6.9±0.25d
0.5%	24.5±0.73b	7.5±0.13c
0.7%	21.4±0.59c	8.3±0.19b
0.9%	19.6±0.48c	9.3±0.17a

Table 3. Mean values of measured parameters with standard errors in salt tolerance experiment of TCS ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests (*statistical analysis could not be performed, because there was only one data)

Concentration, TCS	fresh weight (g/culture)	dry matter content (%/culture)
Control	21.8*	7.9*
0.1%	18.7±0.22a	9.1±0.17a
0.3%	17.7±0.68a	9.4±0.31a
0.5%	18.2±0.36a,b	9.4±0.18b
0.7%	16.5±0.11b,c	10.7±0.09b
0.9%	15.9±0.35c	11.2±0.14b

Table 4. Mean values of measured parameters with standard errors in salt tolerance experiment of SÓS24 ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests (0 means there was no data due to contamination)

Concentration, SÓS24	fresh weight (g/culture)	dry matter content (%/culture)
Control	27.6±0.83a	5.8±0.16c
0.1%	25.7±1.23a,b	6.4±0.21b
0.3%	24.4±1.08b	6.8±0.15b
0.5%	23.5±0.45b	7.3±0.15a
0.7%	23.3±0.64b	7.5±0.10a
0.9%	0	0

Table 5. Mean values of measured parameters with standard errors in salt tolerance experiment of SÓS1 ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Concentration, SÓS1	fresh weight (g/culture)	dry matter content (%/culture)
Control	29.3±0.94a	5.9±0.10e
0.1%	27.6±0.86a,b	6.6±0.24d
0.3%	26.2±1.02b,c	6.9±0.16c,d
0.5%	24.3±0.57c,d	7.4±0.18b,c
0.7%	23.1±0.48d	7.7±0.12b
0.9%	20.7±0.73e	8.5±0.26a

Table 6. Mean values of measured parameters with standard errors in salt tolerance experiment of SÓS2 ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Concentration, SÓS2	fresh weight (g/culture)	dry matter content (%/culture)
Control	27.1±1.60a	5.8±0.08e
0.1%	27.2±0.80a	6.3±0.08d
0.3%	25.5±0.87a,b	7.0±0.14c
0.5%	23.7±0.54b	7.5±0.10b
0.7%	23.8±0.25b	7.5±0.14b
0.9%	21.0±0.44c	8.3±0.13a

JOURNAL of Horticulture, Forestry and Biotechnology
Volume 30(1), 2026, 22-30
https://jhfb.ro/index.php/jhfb
Print ISSN: 2066-1797
Online ISSN: 3045-1876

Table 7. Mean values of measured parameters with standard errors in salt tolerance experiment of GT ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests (0 means there was no data due to contamination)

Concentration, GT	fresh weight (g/culture)	dry matter content (%/culture)
Control	0	0
0.1%	27.2±0.97a	6.8±0.14d
0.3%	21.6±0.52b	8.5±0.18c
0.5%	20.4±0.54b	9.4±0.17b
0.7%	17.8±0.43c	10.7±0.28a
0.9%	16.7±0.62c	11.3±0.34a

Table 8. Mean values of measured parameters with standard errors in salt tolerance experiment of BL ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Concentration, BL	fresh weight (g/culture)	dry matter content (%/culture)
Control	29.8±0.41a	5.6±0.22d
0.1%	29.8±0.48a	5.7±0.09d
0.3%	29.0±0.38a,b	5.9±0.13d
0.5%	27.6±0.91b	6.4±0.16c
0.7%	25.1±0.30c	7.0±0.09b
0.9%	21.5±0.28d	8.2±0.16a

Table 9. Mean values of measured parameters with standard errors in salt tolerance experiment of BCF24 ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Concentration, BCF24	fresh weight (g/culture)	dry matter content (%/culture)
Control	25.7±0.59a	6.6±0.29c
0.1%	23.8±0.82a,b	7.5±0.22b
0.3%	22.9±0.70b	7.9±0.22b
0.5%	22.4±1.00b	7.5±0.23b
0.7%	22.1±0.48b	8.1±0.18b
0.9%	18.8±0.59c	9.2±0.32a

Table 10. Mean values of measured parameters with standard errors in salt tolerance experiment of A1708 ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Concentration, A1708	fresh weight (g/culture)	dry matter content (%/culture)
Control	23.9±0.68a,b	7.9±0.26a,b
0.1%	25.5±0.70a	6.9±0.22b
0.3%	22.3±0.83b,c	6.8±1.38b
0.5%	20.0±0.70d	9.1±0.27a
0.7%	21.2±0.98c,d	8.7±0.27a,b
0.9%	20.3±0.30c,d	8.9±0.11a

Table 11. Mean values of measured parameters with standard errors in salt tolerance experiment of 20SZ ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Concentration, 20SZ	fresh weight (g/culture)	dry matter content (%/culture)
Control	26.7±0.72a	6.6±0.31e
0.1%	26.7±0.77a	6.7±0.36e
0.3%	22.5±0.61b	7.6±0.25d
0.5%	21.1±0.47b	8.4±0.29c
0.7%	19.3±0.25c	9.4±0.40b
0.9%	17.2±0.54d	10.5±0.83a

Table 12. Mean values of measured parameters with standard errors in salt tolerance experiment of 08 ecotype. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Concentration, 08	fresh weight (g/culture)	dry matter content (%/culture)
Control	17.0±0.55a,b	9.8±0.22c
0.1%	17.5±0.39a	9.7±0.24c
0.3%	16.7±0.30a,b,c	10.6±0.20b

0.5%	16.0±0.35b,c,d	10.8±0.42a,b
0.7%	15.5±0.24c,d	11.5±0.21a
0.9%	15.8±0.18d	11.3±0.13a,b

Table 13. Comparison of the ecotypes according to their response to the applied salt stress (values are calculated from the difference of the minimum and maximum fresh or dry weight)

Ecotype	fresh weight losses (g)	Ecotype	dry matter content growth (g)
08	1.7	A1708	1.0
SÓS24	4.3	08	1.5
A1708	5.2	SÓS24	1.7
TCS	5.9	SÓS2	2.5
SÓS2	6.2	SÓS1	2.6
BCF24	6.9	BL	2.6
ES04	8.0	BCF24	2.6
BL	8.3	TCS	3.3
SÓS1	8.6	V2	3.8
20SZ	9.5	20SZ	3.9
GT	10.5	ES04	4.5
V2	11.4	GT	4.5

Table 14. Mean values of measured parameters with standard errors in control cultures. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests (0 means there was no data due to contamination)

type, 0%	fresh weight (g)	type, 0%	dry matter content (%)
V2	31.0±0.64	08	9.8±0.22
BL	29.8±0.41	A1708	7.9±0.26
SÓS1	29.3±0.94	ES04	7.1±0.15
SÓS24	27.6±0.83	20SZ	6.6±0.13
SÓS2	27.1±1.59	BCF24	6.6±0.12
20SZ	26.70±0.72	SÓS1	5.9±0.10
BCF24	25.7±0.59	SÓS24	5.8±0.16
A1708	23.9±.0.69	SÓS2	5.8±0.18
ES04	23.2±0.50	BL	5.6±0.10
08	17.0±0.55	V2	5.5±0.16
TCS	0	TCS	0
GT	0	GT	0

Table 15. Mean values of measured parameters with standard errors in cultures, contained 0.1% NaCl. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

type, 0.1%	fresh weight (g)	Type, 0.1%	dry matter content (%)
V2	30.7±0.85a	08	9.7±0.24a
BL	29.8±0.48a,b	TCS	9.1±0.17b
SÓS1	27.6±0.86b,c	ES04	7.7±0.14c
SÓS2	27.2±0.80c	BCF24	7.5±0.22c
GT	27.2±0.97c	A1708	6.9±0.22d
20SZ	26.7±0.77c	GT	6.8±0.14d,e
SÓS24	25.7±1.23c,d	20SZ	6.7±0.15d,e
A1708	25.5±0.70c,d	SÓS1	6.6±0.24d,e
BCF24	23.8±0.82d,e	SÓS24	6.4±0.21d,e
ES04	22.9±0.76e	SÓS2	6.3±0.08e
TCS	18.7±0.22f	BL	5.7±0.95f
08	17.5±0.39f	V2	5.7±0.05f

Table 16. Mean values of measured parameters with standard errors in cultures, contained 0.3% NaCl. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Type, 0.3%	fresh weight (g)	Type, 0.3%	dry matter content (%)
BL	29.0±0.38a	08	10.6±0.20a
V2	26.5±1.04b	TCS	9.4±0.31b
SÓS1	26.2±1.02b	GT	8.5±0.18c
SÓS2	25.5±0.86b	A1708	8.2±0.47c,d
SÓS24	24.4±1.08b,c	BCF24	7.9±0.22c,d
BCF24	22.9±0.70c,d	ES04	7.7±0.11d
ES04	22.8±0.55c,d	20SZ	7.6±0.11d

20SZ	22.5±0.61c,d	SÓS2	7.0±0.14e
A1708	22.3±0.83c,d	V2	6.9±0.25e
GT	21.6±0.52d	SÓS1	6.9±0.16e
TCS	17.7±0.68e	SÓS24	6.8±0.15e
08	16.7±0.30e	BL	5.9±0.13f

Table 17. Mean values of measured parameters with standard errors in cultures, contained 0.5% NaCl. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Type, 0.5%	fresh weight (g)	Type, 0.5%	dry matter content (%)
BL	27.6±0.91a	08	10.8±0.42a
V2	24.5±0.73b	GT	9.4±0.17b
SÓS1	24.3±0.57b,c	TCS	9.4±0.18b
SÓS2	23.7±1.33b,c	A1708	9.1±0.27b
SÓS24	23.5±0.45b,c	20SZ	8.4±0.12c
BCF24	22.4±0.99c,d	ES04	8.1±0.32c,d
20SZ	21.1±0.47d,e	SÓS2	7.5±0.10d,e
ES04	21.10±0.75d,e	V2	7.5±0.13d,e
GT	20.41±0.54e	BCF24	7.5±0.23d,e
A1708	19.96±0.70e,f	SÓS1	7.4±0.18d,e
TCS	18.22±0.36f	SÓS24	7.3±0.15e
08	16.02±0.34g	BL	6.4±0.38f

Table 18. Mean values of measured parameters with standard errors in cultures, contained 0.7% NaCl. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests

Type, 0.7%	fresh weight (g)	Type, 0.7%	dry matter content (%)
BL	25.1±0.30a	08	11.5±0.21a
SÓS2	23.8±0.25a,b	GT	10.7±0.28b
SÓS24	23.3±0.64b,c	TCS	10.7±0.09b
SÓS1	23.1±0.48b,c	ES04	9.7±0.36c
BCF24	22.1±0.48c,d	20SZ	9.4±0.16c
V2	21.4±0.59d	A1708	8.7±0.27d
A1708	21.2±0.98d	V2	8.3±0.19d
20SZ	19.3±0.25e	BCF24	8.1±0.18d,e
ES04	17.9±0.45f	SÓS1	7.7±0.12e
GT	17.8±0.43f	SÓS2	7.5±0.14e,f
TCS	16.5±0.12f,g	SÓS24	7.5±0.97e,f
08	15.5±0.24g	BL	7.0±0.09f

Table 19. Mean values of measured parameters with standard errors in cultures, contained 0.9% NaCl. Mean values are followed by letters that indicate significantly (p<0.05) different values between treat ments according to ANOVA and Duncan tests (0 means there was no data due to contamination)

Type, 0.9%	fresh weight (g)	Type, 0.9%	dry matter content (%)
BL	21.5±0.28a	ES04	11.6±0.28a
SÓS2	21.0±0.44a	GT	11.3±0.34a
SÓS1	20.7±0.73a	08	11.3±0.13a
A1708	20.3±0.30a	TCS	11.2±0.14a
V2	19.6±0.48a,b	20SZ	10.5±0.34a,b
20SZ	17.2±0.54b,c	V2	9.3±0.17b,c
GT	16.8±0.62b,c	A1708	8.9±0.11c,d
TCS	15.9±0.35c	SÓS1	8.5±0.26c,d
08	15.8±0.18c	SÓS2	8.3±0.13c,d
BCF24	15.6±3.17c	BL	8.2±0.16c,d
ES04	15.2±0.12c	BCF24	7.6±1.55d
SÓS24	0	SÓS24	0

Discussion

In our recent experiment, callus cultures of Giant reed ecotypes were stressed with NaCl *in vitro*. It was applied in their culture medium in six different concentrations (0, 0.1, 0.3, 0.5, 0.7 and 0.9% which corresponds to 0, 1, 2, 5, 7 and 9 g/l, that is 0, 17, 51, 85, 120 and 154 mM, respectively). In the related research publications mainly NaCl was used in salt stress experiments *in vitro*, in the range of 1.7 - 400mM (that is 0.1 – 23.4g/l respectively, which corresponds to 0.01 – 2.3%). Furthermore, in

some researches KCL, Na2SO4, MgSO4 or K2SO4 was used as selective material. 12 ecotypes of Giant reed were treated in our experiment. Differences were detected between ecotypes in their fresh weight and dry matter production under normal (control) conditions. All of the tested ecotypes could grow under high salt conditions, without any browning or necrotic symptom or even cell death which indicates the good salt tolerance capability of Giant reed. But they have differences in growing parameters, such as fresh weight, and dry matter content. All of the ecotypes showed decreased growth under high salt condition (0.9%) and their colour turned pale green in their appearance compared to the fresh green colour of the untreated controls (Figure 1). It suggests there was a reduction in their chlorophyll content. The chlorophyll content of the plant cell is a good indicator of salt stress based on scientific datas. The salt tolerance of the tested ecotypes was also confirmed by their increased dry matter content. Without salt stress, based on the fresh weight, the best growing potential was given by the V2 ecotype, while the greatest dry matter production was of the 08 ecotype. Under moderate salt stress conditions (0.5-0.7% NaCl), the highest growing potential was given by the BL ecotype, while the dry matter production remained the best in case of 08 ecotype. It indicates that BL ecotype is more tolerant against salt stress than V2 ecotype. Under high salt stress condition (0.9% NaCl), the highest growing potential remained the same by the BL ecotype, while there was a change in case of dry matter production where the best result was given by ES04 ecotype. It suggests that ES04 ecotype can tolerate the high salt stress significantly better than BL ecotype. The results of our experiments suggest that giant reed is a promising candidate for the biomass production in salin soil conditions.

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