Effect of Salinity Stress on Ion Accumulation and on the Photosynthetic Activity of a New Energy Plant, *Phalaris arundinacea* Cultivars

Péter Poór[1*], Attila Ördög[1], Barnabás Wodala[1], Péter Borbély[1], Ionel Samfira[2], Monica Butnariu[2], Dóra Hegedűs[1], Katalin Szabó[1], Attila Pécsváradi, Irma Tari[1]:

[1]*Department of Plant Biology, University of Szeged, 6726 Szeged, Közép fasor 52, Hungary*[1]
[2]*Banat University of Agricultural Sciences and Veterinary Medicine ''Regele Mihai I al Romaniei'', Calea Aradului 119, Timișoara 300645, Romania*[2]
e-mail: poorpeti@bio.u-szeged.hu

Abstract

Reed canarygrass (*Phalaris arundinacea*) is a good candidate for bioenergy production in Northern and Middle Europe. The crop is well-adapted to cold and drought stress but its resistance to high salinity has not been revealed in details. In this study the effects of 75 and 150 mM NaCl treatments were investigated on the ion accumulation, water potential changes and photosynthetic activity of three Romanian reed canarygrass genotypes, Tardin, Romanesti diverse and Timpuriu. Since cv. Tardin was able to maintain high K^+ level and relatively low Na^+ concentration in leaf tissues, high stomatal conductance and net CO_2 fixation rate under salt stress and as it could maintain the water potential of tissues at control level, this genotype can be defined as salt tolerant. Salt stress induced significant Na^+ accumulation, very low K^+/Na^+ ratio, and severe reduction in stomatal conductance and photosynthetic activity in the leaf tissues of Timpuriu cultivar, which proved to be sensitive to high salinity.

Introduction

Renewable energy is an important source of energy that reduces the dependence on fossil fuels and emission of greenhouse gases, thus, the use of biofuel feedstock has a great economic impact. There has been increasing interest in the use of perennial grasses as energy crops in Europe since the mid-1980s. The characteristics which make perennial grasses attractive for biomass production are their high yield potential, the high contents of lignin and cellulose of their biomass, and their generally anticipated positive environmental impact [1]. Among those indigenous grasses, which are characterized by regionally high biomass yields and which seem to offer good bioenergy characteristics, reed canarygrass (*Phalaris arundinacea*) was found to be a suitable candidate for Northern Europe [2]. Reed canarygrass belongs to the subfamily *Pooideae* of the *Gramineae* family. It is native to the temperate regions of Europe, including Middle Europe. Reed canarygrass displays a number of advantages, which can be exploited in Northern part of Europe. It is already adapted to short vegetation periods and low temperatures, seed establishment is possible, the biomass has good combustion quality and the plant has broad genetic variability [3].

Phalaris arundinacea is a perennial C3 species growing to 1.5-3 m at a fast rate. It can be cultivated in sandy, loamy and clay soils of broad pH range. Plants have a running root system and form an impenetrable ground cover [4].

Reed canarygrass is one of the main species used in the reed bed system for the water purification treatment of grey water and for pollution control of sewage effluent from municipal and industrial sources. It is one of the best grass species for poorly drained soils and tolerates flooding better than other cool-season grasses. Even though it naturally grows in wet places, it is nevertheless more drought resistant than many other grass species [3].

The content of major elements in reed canarygrass tissues shows considerable differences between different locations and genotypes. Moreover, photosynthetic activity and biomass production of various lines may also show considerable differences. The aim of our work was to reveal these differences after salt stress in photosynthetic activity, biomass production (biomass per one cm^2 leaf area) and ion accumulation of reed canarygrass lines originated in Romania.

Experimental

The salt stress response of three *Phalaris arundinacea* cultivars, Tardin, Timpuriu and Romanesti diverse was investigated in our experiments. The plants were grown in 2.5 kg soil (Bioland Tőzegfeldolgozó Kft., Biatorbágy, Hungary) containing N (20 to 500 mg L^{-1}), P_2O_5 (200 to 500 mg L^{-1}); K_2O (300 to 600 mg L^{-1}), white peat (50%, m/v), black peat (50% m/v), and $CaCO_3$ (2 kg m^{-3}) at pH 7.0. The environmental conditions in the greenhouse were 12/12 h day/night cycle, 25/20 °C day/night temperature, 200 μmol m^{-2} s^{-1} light intensity and 55 to 60% relative humidity. After 12 weeks of pre-culture, the soil was irrigated two times a week with 300 mL of 75 and 150 mM NaCl (stressed plants) for 6 weeks and the control plants received the same irrigation with distilled water. Water potential of the tissues was measured with pressure chamber and the macroelements in plant samples were determined with XSeries II ICP-MS (Thermo Scientific, Bremen, Germany). Net photosynthetic rate (A, μmol fixed CO_2 m^{-2} s^{-1}) and stomatal conductance were measured on fully expanded leaves using a portable photosynthesis system (LI-6400, LI-COR, Inc.; Lincoln, NE), as described by Poór et al. [5].

Results and discussion

Salinity is one of the major abiotic stresses, which reduces plant growth and productivity and can cause programmed cell death in the plant tissues by inducing strong ionic-, osmotic and oxidative stress [6]. Production of reactive oxygen species (ROS) and the failure of ROS-scavenging mechanisms caused by supraoptimal salt concentrations induce a decline in the photosynthetic activity and other biochemical reactions in the tissues, which lead to the degradation of proteins, lipids and DNA and to the decrease of the biomass [7; 8]. Salt tolerant genotypes are able to cope with the ionic stress caused by excess of Na^+ by activating Na^+ exclusion from the cytoplasm.

The K^+ and Na^+ content as well as K^+/Na^+ ratios were very similar values in the control leaves of the three cultivars. The plants were able to maintain potassium contents but accumulated significantly more sodium when they were exposed to salt stress. However, high salinity evoked by 150 mM NaCl resulted in much higher accumulation of Na^+ in the leaf tissues, than medium salt stress, which was exceptionally high in cv. Timpuriu. This led to the reduction in K^+/Na^+ ratios under increasing salt stress, but Timpuriu had the lowest ratio among the three genotypes (Table 1).

Table 1. Changes in the intracellular K^+ and Na^+ contents and the ratio of K^+/Na^+ in the leaves of different *Phalaris* cultivars after exposure to 75 or 150 mM NaCl for six weeks.

Elements (mg gDW^{-1})	Treatments								
	Control			75 mM NaCl			150 mM NaCl		
	Tardin	Romanesti diverse	Timpuriu	Tardin	Romanesti diverse	Timpuriu	Tardin	Romanesti diverse	Timpuriu
K^+	82.7 + 4.7	76.1 + 1.4	76.6 + 2.6	98.1 + 0.8*	65.0 + 0.1***	77.0 + 2.9^{ns}	70.8 + 5.4*	87.9 + 0.9*	74.5 + 0.8^{ns}
Na^+	1.7 + 0.2	1.4 + 0.0	1.7 + 0.2	38.8 + 0.2***	26.0 + 0.2***	35.0 + 3.8***	59.0 + 4.5***	32.5 + 0.0***	111.3 + 1.1***
K^+/Na^+	48.3 + 4.7	52.7 + 0.7	46.6 + 7.2	3.2 + 0.7**	2.4 + 0.0**	2.2 + 0.1**	1.1 + 0.0***	1.8 + 0.8***	0.6 + 0.0***

Results are the average ± SE (n=5). *, **, *** indicate significance levels compared to the untreated control at P < 0.05, 0.01, 0.001, respectively in each time point (Student's t test; ns: not significant).

High salinity induces osmotic stress and a decrease in water potential (Ψ_w) of treated tissues [9]. While cvs Tardin and Romanesti diverse could maintain Ψ_w under salt stress, Timpuriu exhibited a significant decline under high salinity, triggered by 150 mM NaCl (Fig 1A). Increasing salt concentrations induced the closure of stomata and resulted in significant decrease in stomatal conductance, which was most pronounced in Timpuriu cultivar (Fig 1C). Net CO_2 fixation rate, which is a best indicator of biomass production [10], declined significantly in plants exposed to salt stress. Since control plants displayed different basic activities, the decline in the photosynthetic rate was less pronounced in cv Romanesti diverse and most severe in Timpuriu genotype (Fig 1D). As a result, specific leaf area was maintained during salt stress in Tardin genotype while the other two genotypes showed reduction in these parameters under salt stress (Fig 1B).

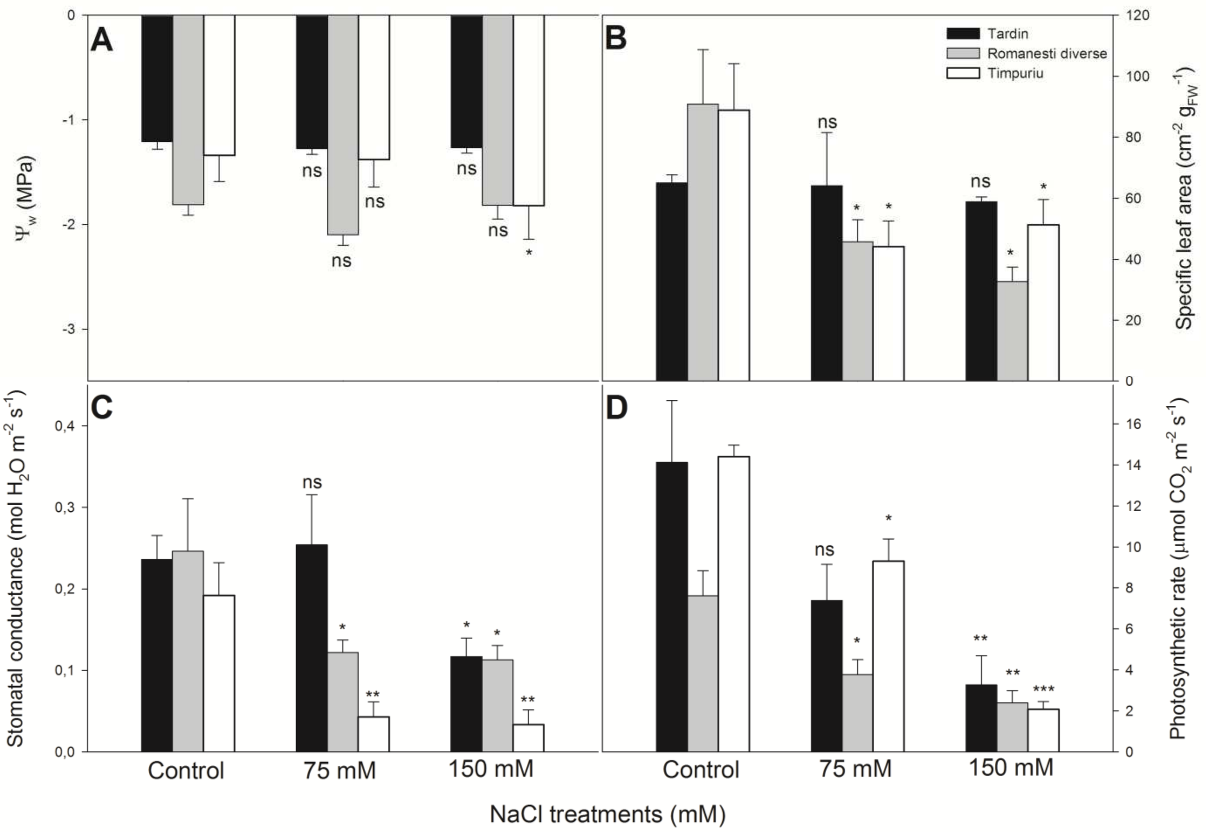

Figure 1. Changes in water potential (A), specific leaf area (B), stomatal conductance (C) and photosynthetic rate (D) in the leaf of different *Phalaris* cultivars after exposure to 75 or 150 mM NaCl for six weeks. Results are the average ± SE (n=5). *, **, *** indicate significance levels compared to the untreated control at P < 0.05, 0.01, 0.001, respectively in each time point (Student's t test; ns: not significant).

Conclusion

Salt stress resulted in higher Na^+ levels and lower K^+/Na^+ ratio in the leaf tissues of reed canarygrass genotypes, these parameters of Timpuriu cultivar overtopped the changes observed in cvs Tardin and Romanesti diverse.

On the basis of the photosynthetic parameters, stomatal conductance, water potential and specific leaf area changes we can conclude that cv. Tardin proved to be most tolerant to salinity stress among the three genotypes of reed canarygrass, while cv. Timpuriu was the most sensitive cultivar.

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