CAN *Setaria viridis* (A 10.1) BE USED AS MODEL PLANT FOR VALIDATION OF GENES FOR SALINITY TOLERANCE?

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ABSTRACT: Soil salinity is one of the main abiotic stresses limiting growth and productivity of plants. Salinity tolerance can be achieved by heterologous expression or gene editing strategies. *Setaria viridis* has been used as model plant in fast proof-of-concept studies aiming gene function validation. To use a plant as a model for studying a particular type of stress, one must show that it is susceptible (not tolerant) to that stress. Here we aimed to perform physiological analyses to prove that access A 10.1 of *S. viridis* can be used for the validation of genes for salt tolerance. For this, setaris seeds were germinated in culture medium (MS ½ strength, Phytagel 0.2%, and pH 5.8) under a photoperiod of 16/8 hours (light/dark), 25 ±2 °C and light intensity of 150 µmol $m^{-2}s^{-1}$. A week later the seedlings were transferred to a substrate and maintained under a photoperiod of 16/8 hours (light/dark), 25 ±2°C, 60% air humidity, and light intensity of 500 µmol $m^{-2}s^{-1}$. Fourteen days after sowing, plants were submitted to different NaCl concentrations (0, 2, 4, 6, 8 and 10 g / 100 g of substrate) for a period of 12 days. Results showed reduction in the plants biomass which was directly proportional to the increase in the salt amount in the substrate up to 6 g/dm³. The plants submitted to salt contents above 8 g of NaCl did not survived to stress. All salt stressed plants showed a reduction in the rates of stomatal conductance and transpiration. However, only plants submitted to 6 g NaCl had a reduction in the net assimilation rate of CO_2 and in the chlorophyll content.

KEYWORDS: Salinity, tolerance, Setaria.

Setaria viridis (A 10.1) PODE SER UTILIZADA COMO PLANTA MODELO PARA VALIDAÇÃO DE GENES DE TOLERÂNCIA A SALINIDADE?

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RESUMO: A salinidade do solo é um dos principais estresses abióticos que limitam o crescimento e a produtividade das plantas. A tolerância à salinidade pode ser obtida através da expressão heteróloga ou estratégias de edição gênica. *Setaria viridis* tem sido utilizada como planta modelo em estudos de prova rápida de conceito, visando a validação da função de genes. Para utilizar uma planta como modelo no estudo de um determinado stresse, deve-se mostrar se ela é suscetível (não tolerante) a esse estresse. Neste trabalho objetivamos realizar análises fisiológicas para comprovar que o acesso A 10.1 de *S. viridis* pode ser utilizado para a validação de genes de tolerância à salinidade. Para isso, as sementes de setaria foram germinadas em meio de cultura (MS ½ força, Phytagel 0,2% e pH 5,8) sob fotoperíodo de 16/8 horas (luz / escuridão), 25 ± 2 ° C e intensidade de luz de 150 μmol m^{-2} s^{-1}. Após uma semana, as mudas foram transferidas para um substrato e mantidas sob um fotoperíodo de 16/8 horas (luz / escuro), 25 ± 2 ° C, 60% de humidade do ar e intensidade de luz de 500 μmol m^{-2} s^{-1}. Quatorze dias após a semeadura, as plantas foram submetidas a diferentes concentrações de NaCl (0, 2, 4, 6, 8 e 10 g / 100 g de substrato) por um período de 12 dias. Os resultados mostraram redução na biomassa das plantas, que foi diretamente proporcional ao aumento da quantidade de sal no substrato até 6 g / dm^3. As plantas submetidas a teor de sal superior a 8 g de NaCl não sobreviveram ao estresse. Todas as plantas salinizadas mostraram uma redução nas taxas de condutância estomática e transpiração. No entanto, apenas as plantas submetidas a 6 g de NaCl tiveram uma redução na taxa de assimilação líquida de CO_2 e no teor de clorofila.

PARAVRAS-CHAVE: Salinidade, tolerância, Setaria.

INTRODUCTION

Abiotic stresses has caused great losses to the world agriculture, and salinity is one of the most troublesome of those stresses (MUNNS; TESTER, 2008). The use of saline water for irrigation and the excessive use of fertilizers are the main causes of soil salinity (PEDROTTI, CHAGAS; RAMOS, 2015). According to the FAO (2017), 19.5% of the current 230 million hectares of irrigated land are affected by excess salts.

Salinity reduces the osmotic potential of the soil and causes the water retention forces in the soil to be greater than the forces responsible for the process of water absorption by the roots (DIAS; BLANCO, 2010), and this reduction of the osmotic potential leading initially to a stress due to water deficit. When accumulated, these salts can reach toxic levels and cause premature

senescence of the older leaves. The reduction of the photosynthetic leaf area of the plant directly affects its growth and productivity (MUNNS, 2002).

Salinity-tolerant plants are able to harbor larger amounts of ions in the cytoplasm, reducing the toxic effect of the plant and allowing a more favorable osmotic gradient for water uptake by the roots (GUPTA; HUANG, 2014). This tolerance may be related to single responsive genes, linked to biochemical and molecular mechanisms to tolerate saline stress, which would be absent in sensitive plants (PARIHAR et al., 2015).

Due to favorable biological aspects, *Setaria viridis* has been studied to become a model plant for the validation of candidate genes in monocots (JIANG; BARBIER; BRUTNELL, 2013). However, it is necessary that in addition to characteristics inherent to a model plant, *S. viridis* must lack or be susceptible (or intolerant) to target traits. Thus, intolerance to salinity can be verified through the evaluation of parameters such as the rate of photosynthesis and transpiration, intracellular CO_2 concentration, chlorophyll concentration, leaf area, biomass, root morphology, among others (FERRAZ et al., SILVA et al., 2011).

The objective of this study was to determine the degree of susceptibility of *S. viridis* (access A10.1) to the excess of salts, seeking to subsidize the studies related to the use of this species as a model plant for the validation of putative tolerance genes to saline stress.

MATERIAL & METHODS

Setaria viridis (access A10.1) seeds were soaked in sulfuric acid (1000 μM) for 15 minutes, in order to break the dormancy. After washing, they were disinfected in solution with sodium hypochlorite (2%) and Tween 20® (0.1 %). Half strengthen Murashige & Skoog (MS) medium was used for seed germination (MARTINS et al, 2015). Twenty seeds were sowed per petri dish, and kept in a Conviron® growth chamber (Adaptis 1000TC model) under photoperiod of 16:8 hours light/dark, temperature of 25 ± 2°C, and light intensity of 150 Mmol $m^{-2}s^{-1}$.

Seven days after sowing, seedlings were transplanted individually into 0.2 L pots containing 100 g of a substrate (soil, commercial substrate, and vermiculite - 2: 1: 1; v / v / v), and transferred to a Conviron growth chamber Mod. PGW40, kept under the same previous conditions, except for light intensity (500 μmol $m^{-2}s^{-1}$).

Fourteen days after sowing, plants were submitted to different treatments (T) by the addition of NaCl: T1 (2 g/dm^3), T2 (4 g/dm^3), T3 (6 g/dm^3), T4 (8 g/dm^3), T5 (10 g/dm^3), in addition to the control (C), without NaCl. Ten plants per treatment and one pot without plant

were used for each salt concentration in order to estimate the amount of salt in solution that could be removed by the plants. Throughout the experiment, the replenishment of water in each pot was performed so that it returned to the initial weight in field capacity. The assay was conducted for 12 days.

The evaluation of plants under saline stress occurred by the measurement of the gas exchange (IRGA - LICOR model 6400XT), in the youngest completely expanded leaf. The chlorophyll concentration index (portable chlorophyll meter - model CCM-200, Opti-Science) was also evaluated. Measurements were taken at the end of the experiment (or 12 days after stress onset). Dry mass was obtained after 52 days of transplanting, in an oven at 72°C, with forced ventilation until a constant mass was obtained.

RESULTS & DISCUSSION

There was a reduction in the aerial parts and root biomass of the *S. viridis* plants as the amount of NaCl was increased in the substrate. All plants died in the doses of 8 and 10 g/dm³ of NaCl (Figure 1 and Table 1). Plant mortality may be related to water stress caused by the osmotic effect, as well as the reduction of nutrient uptake by ionic competition generated by high salinity (FEIJÃO et al., 2013).

Tolerant plants can maintain reasonable photosynthetic rates even under unfavorable conditions (OLIVEIRA JUNIOR et al., 2016). The reduction of the photosynthetic rate by saline stress can occur due to dehydration of cell membranes, which reduces the permeability of CO_2, closure of stomata and consequent reduction of CO_2 supply, leaf senescence, change in enzyme activity, such as, for example, the inhibition of photosynthetic carbon fixation activity and salt toxicity, which accumulate in the chloroplasts (ESTEVES; SUZUKI, 2008; SONG et al., 2005).

In the plants of *S. viridis* which survived the saline stress, gas exchange measures (Table 2) showed that NaCl caused reduction in the stomatal conductance and transpiration rates which was roughly proportional to the level of salt in the substrate. However, only plants subjected to 6 g/cm3 of NaCl suffered a statistically significant reduction in the net CO_2 assimilation rate. It is interesting to notice that the control plants presented the highest values for the internal CO_2 concentration (IC). On the other hand, the lower values of IC were observed in the plants submitted to the lower amounts of NaCl (2 g/dm3). From that salt level, as the amount of NaCl was increased in the substrate, there was also an increase in IC, so that in plants under 6 g/cm3 of NaCl the IC values did not differ statistically from the control.

The reduction of photosynthesis as a function of salinity has been attributed to limitations of stomatal and non-stomatal origin (MUNNS; TESTER, 2008; PARIHAR et al., 2015; SOUZA et al., 2011). In *S. viridis*, there was a clear drop in the stomatal conductance and transpiration rates which was enhanced by the increase of saline levels in the substrate (Table 2). This indicate closure of the stomata to avoid water loss. However, the decrease in the net CO2 assimilation rate was only observed when the salt level in the substrate reached 6 g/d m^3. In addition, it was observed that the net assimilation rate of CO_2 only fell significantly by 6 g/dm^3, and that the IC fell by 2 and 4 g/dm^3 and increased by 6 g/dm^3, being that this last one did not differ statistically from the control.

Taken together, these results suggest that the maintenance of CO2 assimilation rate in the S. viridis plants subjected to salt concentrations of 2 and 4 g/dm^3 was possible thanks to the use of internal CO_2 that was already in the leaf. Therefore, the internal CO2 levels in the plants submitted to these salt concentrations was reduced. The decrease in CO2 assimilation rates at the dose of 6 g/dm3 indicates that there was restriction to CO2 fixation. Consequently, internal CO2 levels increased, despite the stomatal restriction at the entrance of CO2.

Similar responses were found for salt-grass (*Atriplex nummularia*), a halophyte plant highly tolerant to salt stress. The decrease in net CO_2 assimilation rates coincided with the increase of the internal CO2 concentration, indicating an inhibition of photosynthetic carbon fixation. However, because it is a salinity-tolerant plant, there was a small variation of the values of IC in the last evaluated periods and return of the photosynthetic CO_2 assimilation, thus depicting a period of acclimatization to the imposed stress (OLIVEIRA JUNIOR et al., 2016); which was not observed for *S. viridis*, since measures after the 12th day of stress were not performed.

In addition to its effects on gas exchange, the increase in saline concentration on the substrate also negatively affected the concentration index of chlorophyll in plants of *S. viridis*, but only at the dose of 6 g/dm3. Similar results were found in *O. sativa* (AMIRJANI, 2011; CHUTIPAIJIT; CHA-UM; SOMPORNPAILIN, 2011) and *Vigna radiate* (SAHA; CHATTERJEE; BISWAS, 2010) subjected to salinity stress. These results may be related to the deterioration of the chloroplast membrane (MANE et al. 2010).

The drastic fall in the leaf area and also in the total length of the roots shows that the plants did not develop properly when subjected to saline stress, which shows a certain degree of sensitivity of this access. These results indicate, therefore, that a prospective gene aimed at improving these characteristics may, in this context, be validated in *S. viridis*.

CONCLUSION

The results presented here indicate a certain sensitivity of *S. viridis* to salinity stress. It is believed that this study may contribute to endorse this species as a model plant for the validation of putative salinity tolerance genes.

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FIGURES AND TABLES

Figure 1. The effects of the NaCl concentration on the development of the aerial part of *Setaria viridis* (access A10.1) plants at 12 after the onset of the salinity stress. Digital RGB images of plants: 1 (Control - 0.0 g), 2 (T1 - 0.2 g), 3 (T2 - 0,4 g), 4 (T3 - 0.6 g), 5 (T4 – 0.8 g) and 6 (T5 - 1.0 g).

Table 1. Effect of the NaCl concentration on the substrate on the aerial part and root biomass of *Setaria viridis* (access A10.1) plants at eight days after the onset of the salinity stress.

NaCl (g/dm³)	Aerial part (g)	Root (g)
0	2.97 ± 0.29	1.01 ± 0.08
2	1.68 ± 0.01	0.56 ± 0.06
4	1.28 ± 0.07	0.44 ± 0.06
6	0.78 ± 0.04	0.19 ± 0.05
8	0.15 ± 0.04	0.07 ± 0.01
10	0.14 ± 0.01	0.05 ± 0.00

Table 2. The effects of the NaCl concentration on the variables of gas exchange in *Setaria viridis* (access A10.1) plants at 12 days after the onset of the salinity stress.

NaCl (g/dm³)	*A*	*gs*	*E*	*Ci*
0	28.83a	0.242a	3.62 a	170.86a
2	28.89a	0.188b	2.95 b	118.51c
4	26.11ab	0.184bc	2.86 bc	141.45b
6	21.57b	0.15c	2.47c	151.36ab
CV%	12,75	13,13	11,12	11,45

Data followed by the same letter on the column do not differ statistically from each other by Tukey's test ($p \leq 0.05$). *A*: Net assimilation rate of CO_2 (μmol of CO_2 m^{-2} s^{-1}); *gs*: stomatal conductance rate to water vapor (mol of H_20 m^{-2} s^{-1}), *E*: transpiration rate (mmol of H_2O m^{-2} s^{-1}); IC: intracellular concentration of CO_2 (μmol of CO_2 mol^{-1}).

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