Low salt stress affects physiological parameters and sugarcane plant growth

Flávio José Rodrigues Cruz*, Domingos da Costa Ferreira Júnior, Durvalina Maria Mathias dos Santos

São Paulo State University (UNESP), Faculty of Agricultural and Veterinary Sciences, Câmpus Jaboticabal, São Paulo, Brazil

*Corresponding author: fjrcpp@outlook.com

Abstract

Sugarcane is a renewable source for biofuel production and widely grown in tropical regions of the world. However, its cultivation might be limited in regions with soils affected by high level of soluble salts. This work was carried out aiming to evaluate the response of the sugarcane cultivar IAC91-1099 to low soil salinity under pot conditions, based on the hypothesis that low salt stress affects sugarcane growth. The experimental design was completely randomized, formed by the control treatment and 800 mg Na kg⁻¹ soil. After twenty-five days of transplanting, the plants were submitted to the treatments and after thirty days under saline stress, the plants were harvested and the variables evaluated. Plants under salt stress presented higher Na content and Na/K ratio, and lower K content. Water status of sugarcane plants was impaired due to salt stress, expressed by lower values of stomatal conductance, relative water content and water potential. Na accumulation induced a decline on plant growth and higher electrolyte leakage, with damage to the photochemical apparatus of photosynthesis. Although sugarcane is considered a moderately salt tolerant crop, cultivar IAC91-1099 was sensitive to the low salt stress studied, not being recommended for cultivation in regions with pronounced soluble salt contents.

Keywords: salinity; osmotic stress; relative water content; chlorophyll fluorescence; growth.

Abbreviation: Fo_initial fluorescence, Fm_maximum fluorescence, Fm, Fv/Fm_photochemical efficiency of the photosystem II, ΔΨwpd_predawn water potential, ΔΨmid-day mid-day water potential, RWC_relative water content, EL_electrolyte leakage, gs_stomatal conductance.

Introduction

Despite of being native from Asia and grown in several countries, sugarcane became prominent in Brazil due to its utilization for ethanol and sugar production, as this country is currently the largest producer of this crop (FAO, 2017). It is estimated that, in the agricultural year of 2016/2017, the area planted with sugarcane will be 9,073.7 ha, corresponding for a production of 691 million tons of culms (CONAB, 2017). However, its cultivation is limited by several abiotic factors, among which is the high concentration of soluble salts in soils, despite this crop being considered a glycophyte specie, i.e., moderately sensitive to salt stress (Patade et al., 2008).

Soil salinity is a major limiting factor to crop production, affecting millions of hectares worldwide (Munns, 2005). It is estimated that 6% of the arable land of the world has some degree of salinity caused by natural factors, like wind-borne sea-salt deposition and rock weathering, or by anthropic action, that through deforestation induces higher evaporation of underground water and consequent deposition of soluble salts in superficial soil horizons (Almeida et al., 2017). On the other hand, the excessive use of fertilizers with high saline index can increase the levels of salts in the soil. The high salinity of fertilizers such as KCl compromises the growth and distribution of the root system as well as the absorption of water and nutrients (Marschner, 1997).

In plants, salt stress occurs due to excessive absorption of toxic ions, mainly sodium (Na), leading to physiological disorders that decrease plant growth and development. These disorders are results of osmotic stress, due to lower soil water potential caused by high salt accumulation in the root zone, and of ionic stress, consequence of the Na toxicity to metabolic processes. Furthermore, salt stress also impairs photosynthesis through stomatal and non-stomatal restrictions (Flexas et al., 2004), limitation of the photochemical apparatus (Lawlor and Cornic, 2002) and damage to photosynthetic pigments (Nawaz and Ashraf, 2010), and membranes prompting electrolyte leakage (Patade et al., 2011).

Several studies report the damaging effect of saline stress on plant growth due to physiological disorders resulting from the absorption of toxic ions such as Na⁺. For example, the HSF-240 sugarcane genotype saline stress-sensitive showed considerable reduction in shoot dry matter and leaf area after saline stress exposure of 8 and 12 dS m⁻¹ for thirty days. Coincidentally, this cultivar absorbed greater amount of Na⁺ in relation to K (Wahid and Ghazanfar, 2006). In another study with seedlings of seven sugarcane cultivars, saline stress of 200 mM NaCl significantly affected growth in
parallel to the higher accumulation of Na in leaf tissues (Chauum et al., 2012). In this way, plants sensitive to salt stress show a close relationship between growth reduction and higher Na accumulation compared to K. The salt tolerant plants develop mechanisms to deal with excessive Na, like compartmentalization in vacuoles or even exclusion through specific membrane transporters (Almeida et al., 2017). Yet, the physiological responses of glycophytes species exposed to long-term salt stress still lack deeper comprehension, particularly regarding ionic and water status adjustment, as these are key elements involved in tolerance to this abiotic condition. Considering that sugarcane is moderately tolerant to salt stress, this work was carried out to evaluate the hypothesis that young sugarcane plants under long-term exposure to low salt stress have their growth and physiological traits impaired. This study aimed to evaluate the growth and physiological parameters of sugarcane plants exposed to prolonged periods of low salt stress.

Results

**Sodium and potassium leaf content**

Salt stress induced substantial modifications in foliar Na and K content. Na content in salt stressed plants was 98.5% higher ($P < 0.05$) in comparison to the control group (Fig 1A). On the other hand, leaf K concentration was 37% lower ($P < 0.05$) when sugarcane plants were exposed to stress (Fig 1B). Na/K ratio (Figure 1C) was increased ($P < 0.05$), as observed for Na contents in plants under salt stress (Fig 1A).

**Stomatal conductance and chlorophyll fluorescence measurement**

Stomatal conductance had a 48.3% reduction ($P < 0.05$) in plants under salt stress compared to control plants (Figure 2A). Similarly, there was a 14.6% decrease ($P < 0.05$) in relative water content of salt stressed plants (Fig 2B). Both $\Psi_{pd}$ (Fig 2C) and $\Psi_{mm}$ (Figure 2D) assessments reflected a harmful effect of salt stress, with values 154.3 and 222% lower ($P < 0.05$), respectively, compared to the control plants.

Chlorophyll fluorescence was significantly modified ($P < 0.05$) in young sugarcane plants under low NaCl soil concentrations (Figures 3A, B and C). Initial fluorescence increased 21.1% ($P < 0.05$) in plants under salt stress in comparison to check treatment (Figure 3A). Furthermore, maximum fluorescence (Fm) and photochemical efficiency of the photosystem II (Fv/Fm) were 30.6 and 10.8% lower ($P < 0.05$) in stressed plants (Figures 3B and 3C). Relative chlorophyll content estimated by SPAD index (Figure 3D) also was 13.8% lower ($P < 0.05$) in the saline condition.

**Plant growth measurement and electrolyte leakage determination**

In most evaluations, salt stress impaired ($P < 0.05$) sugarcane growth (Figures 4A, 4B, C, D, E and F). Plant height and leaf area presented a decrease of 29 and 44% ($P < 0.05$), respectively, compared to non-stressed plants (Figures 4A and 4B). Regarding fresh and dry weight, it is noticeable that salt stress led up to a decline of these parameters for the entire plant ($P < 0.05$), reaching values of 48.5 and 57.8% for shoots (Figure 4C and D) and 46.2% and 51.9% for roots (Figure 4E and F) in comparison to check treatment. Electrolyte leakage was also higher in salt stressed plants, with a value 43.2% higher ($p < 0.05$) than plants not exposed to saline condition (Figure 5).

Discussion

Salt stress in young sugarcane plants led to significant Na accumulation in leaves (Figure 1A). Simultaneously, there was less K accumulation and higher Na/K ratio (Figures 1B and C). Since Na is passively absorbed by roots and its efflux requires energy consumption (Almeida et al., 2017), there was an ionic imbalance between Na and K, as highlighted by the high Na/K ratio in the present study (Figure 1C). It has to be considered that Na impairs K absorption, mainly when Na is in higher concentration in the environment. This occurs because specific K transporters are inhibited by Na (Hirsch et al., 1998; Fuchs et al., 2005; Nieves-Cordones et al., 2010). Through the present results, it is evident that the studied cultivar (IAC911099) is inefficient in maintaining high K rates in foliar tissues, a recurrent strategy of glycophyte plants to tolerate salt stress, since K is the main inorganic compound related to osmotic adjustment in stressed plants (Wu et al., 1995). It is essential to preserve a satisfactory Na/K ratio in plants to allow K accumulation and plant growth under the abiotic constraints (Sentenac et al., 1992; Tas and Basar, 2009).

The presence of soluble salts in soil implies in lower water potential and water transport in the soil-plant-atmosphere system. This response pattern was observed in young sugarcane plants, represented by the assessed values of gs, RWC, $\Psi_{pd}$ and $\Psi_{mm}$ (Figures 2A, B, C e D). Although gs decrease is an alternative against water loss and tissue dehydration (Chaves et al., 2009), in the present study water loss control trough reduced gs, evidenced by the lower RWC, $\Psi_{pd}$ and $\Psi_{mm}$ values (Figures 2B, C and D), resulted in decreased young sugarcane plants growth (Figure 4A, B, C, D and F). These results are corroborated by the high correlation ($p < 0.05$) between growth and water status parameters, as well by the correlation between Na and the parameters related to water status and growth (Table 1). This way, it is suggested that water loss regulation by gs might have restricted CO$_2$ diffusion, since Fv/Fm was decreased, in parallel with lower chlorophyll content estimated by SPAD index (Figure 3D), causing impaired growth of young sugarcane plants. Lower gs and modifications in the photochemical apparatus of photosynthesis, which were registered in young sugarcane plants exposed to salt stress, are characteristics of the photosynthesis inhibition through stomatal and non-stomatal pathways (Chaves et al., 2009).

Salt stress had a deleterious effect on the chlorophyll fluorescence parameters (Figures 3A, 3B and 3C) as a consequence of lower contents of these pigments estimated using the SPAD index (Figure 3D) and reduced gs (Figure 2A). The chlorophyll fluorescence decline due to salinity can be considered an indirect effect, because stomatal closure promotes lower CO$_2$ influx, impairment of the electron transport chain and overproduction of reducing power of NADPH, which probably increases superoxide radical generation in PS II, damaging the photochemical apparatus.
Table 1. Chemical and physical properties of soil studied.

<table>
<thead>
<tr>
<th>pH</th>
<th>OM (%)</th>
<th>Na</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>0.3</td>
<td>0.3</td>
<td>16.5</td>
<td>1326</td>
<td>420</td>
<td>144</td>
<td>49.8</td>
<td>4.2</td>
<td>46.0</td>
</tr>
</tbody>
</table>

Fig 1. Sodium (A) and potassium content (B), and sodium/potassium ratio. Values are means ± SD (n = 10). Different letters indicate significant differences at p < 0.05. First column indicates control treatment and second 800 mg Na kg⁻¹ soil, respectively.

Table 2. Pearson correlation analyses between evaluated variables. Values are significant at P < 0.05 (n = 10).

<table>
<thead>
<tr>
<th></th>
<th>gs</th>
<th>CRA</th>
<th>ψwₚ</th>
<th>ψwᵣ</th>
<th>EL</th>
<th>Fo</th>
<th>Fm</th>
<th>Fv/Fm</th>
<th>SPAD</th>
<th>LA</th>
<th>SFM</th>
<th>SFR</th>
<th>SDM</th>
<th>RDM</th>
<th>Heigh</th>
<th>Na</th>
<th>K</th>
<th>Na/K</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>-0.88</td>
<td>-0.81</td>
<td>-0.74</td>
<td>-0.40</td>
<td>0.69</td>
<td>0.87</td>
<td>0.80</td>
<td>0.91</td>
<td>0.80</td>
<td>0.75</td>
<td>0.87</td>
<td>0.73</td>
<td>0.64</td>
<td>-0.92</td>
<td>0.84</td>
<td>-0.90</td>
</tr>
<tr>
<td>CRA</td>
<td>-</td>
<td>1.00</td>
<td>-0.84</td>
<td>-0.82</td>
<td>-0.76</td>
<td>-0.27</td>
<td>0.78</td>
<td>0.72</td>
<td>0.73</td>
<td>0.85</td>
<td>0.71</td>
<td>0.69</td>
<td>0.75</td>
<td>0.75</td>
<td>0.58</td>
<td>-0.86</td>
<td>0.85</td>
<td>-0.89</td>
</tr>
<tr>
<td>ψwₚ</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.91</td>
<td>0.79</td>
<td>-0.51</td>
<td>0.64</td>
<td>0.78</td>
<td>-0.70</td>
<td>-0.89</td>
<td>-0.83</td>
<td>-0.77</td>
<td>-0.84</td>
<td>-0.73</td>
<td>-0.58</td>
<td>-0.94</td>
<td>0.85</td>
<td>-0.93</td>
</tr>
<tr>
<td>ψwᵣ</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.69</td>
<td>-0.51</td>
<td>0.71</td>
<td>0.85</td>
<td>-0.80</td>
<td>-0.83</td>
<td>-0.93</td>
<td>-0.83</td>
<td>-0.92</td>
<td>-0.85</td>
<td>-0.63</td>
<td>-0.94</td>
<td>0.88</td>
<td>-0.93</td>
</tr>
<tr>
<td>EL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.43</td>
<td>-0.53</td>
<td>-0.65</td>
<td>-0.60</td>
<td>-0.68</td>
<td>-0.63</td>
<td>-0.60</td>
<td>-0.69</td>
<td>-0.68</td>
<td>-0.41</td>
<td>0.78</td>
<td>-0.80</td>
<td>0.79</td>
</tr>
<tr>
<td>Fo</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>-0.24</td>
<td>-0.55</td>
<td>-0.41</td>
<td>-0.32</td>
<td>-0.47</td>
<td>-0.59</td>
<td>-0.50</td>
<td>-0.43</td>
<td>-0.30</td>
<td>0.52</td>
<td>-0.48</td>
<td>0.49</td>
</tr>
<tr>
<td>Fm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.84</td>
<td>0.65</td>
<td>0.66</td>
<td>0.71</td>
<td>0.67</td>
<td>0.77</td>
<td>0.73</td>
<td>0.67</td>
<td>-0.77</td>
<td>0.66</td>
<td>-0.77</td>
</tr>
<tr>
<td>Fv/Fm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.80</td>
<td>0.79</td>
<td>0.89</td>
<td>0.84</td>
<td>0.93</td>
<td>0.81</td>
<td>0.81</td>
<td>0.72</td>
<td>-0.90</td>
<td>0.81</td>
<td>-0.88</td>
</tr>
<tr>
<td>SPAD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.78</td>
<td>0.76</td>
<td>0.70</td>
<td>0.80</td>
<td>0.75</td>
<td>0.75</td>
<td>0.62</td>
<td>-0.85</td>
<td>0.85</td>
<td>-0.85</td>
</tr>
<tr>
<td>LA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.78</td>
<td>0.67</td>
<td>0.77</td>
<td>0.67</td>
<td>0.60</td>
<td>-0.90</td>
<td>0.87</td>
<td>-0.91</td>
<td></td>
</tr>
<tr>
<td>SFM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.83</td>
<td>0.91</td>
<td>0.88</td>
<td>0.75</td>
<td>-0.90</td>
<td>0.85</td>
<td>-0.89</td>
<td></td>
</tr>
<tr>
<td>SFR</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.92</td>
<td>0.85</td>
<td>0.71</td>
<td>-0.86</td>
<td>0.80</td>
<td>-0.86</td>
<td></td>
</tr>
<tr>
<td>SDM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.89</td>
<td>0.71</td>
<td>-0.94</td>
<td>0.85</td>
<td>-0.92</td>
<td></td>
</tr>
<tr>
<td>RDM</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>0.68</td>
<td>-0.86</td>
<td>0.80</td>
<td>-0.84</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>-0.72</td>
<td>0.71</td>
<td>-0.74</td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>-0.94</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td>-0.96</td>
<td></td>
</tr>
<tr>
<td>Na/K</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>
Fig 2. Stomatal conductance (A), relative water content (B), predawn water potential (C) and midday water potential (D). Values are means ± SD (n = 10). Different letters indicate significant differences at $p < 0.05$. First column indicates control treatment and second 800 mg Na kg$^{-1}$ soil, respectively.

Fig 3. Initial fluorescence (A), maximum fluorescence (B), maximum quantum efficiency of photosystem II (C), and chlorophyll content through SPAD index (E). Values are means ± SD (n = 10). Different letters indicate significant differences at $p < 0.05$. First column indicates control treatment and second 800 mg Na kg$^{-1}$ soil, respectively.
Fig 4. Plant height (A), leaf area (B), shoot (C) and root fresh matter (D), shoot (E) and root dry matter (F). Values are means ± SD (n = 10). Different letters indicate significant differences at $P < 0.05$. First column indicates control treatment and second 800 mg Na kg\(^{-1}\) soil, respectively.

Fig 5. Leaf electrolyte leakage. Values are means ± SD (n = 10). Different letters indicate significant differences at $P < 0.05$. First column indicates control treatment and second 800 mg Na kg\(^{-1}\) soil, respectively.
of photosynthesis (Miller et al., 2010). On the other hand, lower Fo (Figure 3A) indicates a decreased photon flux from the collector system to the PSII reaction center (Baker and Rosenqvist, 2004), which can be a consequence of lower chlorophyll in salt-stressed plants (Figure D). There was a positive correlation between Fo and Na content \((r = 0.52)\), followed by a high negative correlation between Na and Fm \((r = -0.77)\) and between Na and Fv/Fm \((r = -0.9)\) (Table 2), revealing a harmful effect of Na on the photochemical apparatus even in low-salinity conditions (Richards, 1954).

Cell membrane is one the main targets of stress in plants, and is generally accepted that the maintenance of its integrity and stability under drought or salt stress is the major component in osmotic tolerance in plants (Bajji et al., 2001). In this work, it was reported that salt stress caused a significant electrolyte leakage (Figure 5), along with higher Na foliar accumulation in salt-stressed plants (Figure 1A), which had a high correlation with the former parameter \((r = 0.78)\). Salt stress induced higher electrolyte leakage due to cell membrane disruption caused by excessive Na accumulation (Figure 1A), as observed in maize (Kaya et al., 2013) and rice (Hoang et al., 2015) under salt stress.

In this study, the sugarcane cultivar IAC911099, in its early development, was proven susceptible to low salt stress due to the negative impacts in its growth, despite of the fact that sugarcane is considered moderately tolerant to salinity (Brady and Weil, 2013). The growth decline is followed by decreased water status, imbalanced ion contents and impaired chlorophyll fluorescence parameters. Our results are supported by the strong correlation between the leaf Na content and growth, water status, chlorophyll fluorescence and electrolyte leakage assessments. It can be concluded that sugarcane cultivar IAC911099 is salt-sensitive even in low salinity conditions. This way, sugarcane cultivation in salt-affected environments, even though this crop is considered moderately tolerant to salt stress, should consider the genotypic variation on decision making, as some cultivars, as the one evaluated in this work, are highly sensitive to this environmental constraint.

Materials and methods

Plant material, growth conditions, soil salinity and treatments

The present study was carried out at the Faculty of Agricultural and Veterinary Sciences - São Paulo State University, Campus Jaboticabal, Brazil, under greenhouse conditions with average temperature and relative humidity of 30.2 °C and 40.7%, respectively, from February to April of 2013. 10 cm-length mini cuttings of the sugarcane cultivar IAC911099 were planted in sand (previously autoclaved) and irrigated with deionized water for 20 days. After this period, sugarcane seedlings were transplanted to 8 dm³ pots filled with Hapludox soil collected from the depth of 0-20 cm. The results of soil characterization are shown in Table 1 with the following properties: pH 5.7; 3.0% of organic matter; 0.3 mg/kg Na; 16.5 mg/kg P; 1326 mg/kg K; 420 mg/kg Ca; 144 mg/kg Mg; 341 g/kg clay, 28 g/kg silt, 310 g/kg fine sand, 321 g/kg coarse sand; and density of 1.24 kg/L. Soil salinization was induced according to Raji et al. (2001). For this purpose, a preliminary test was performed in triplicate and consisted of salinization of 0.1 dm³ of soil with 30 mL of saline solution at two concentrations (0, control; and 800 mg Na/kg soil, salt stress. Salt stress corresponding to 2.032 g NaCl per kg soil), using NaCl as the salt source (Sigma-Aldrich). After drying for 24 h in a greenhouse environment (where the study was conducted), the samples were sieved (2 mm mesh) and a 10 cm² fraction was collected, to which 50 mL of deionized water was added at the proportion of 1:5 (soil:water). After stirring for 15 min and decanting at room temperature, the supernatant was filtered on medium-coarse texture filter paper. Electrical conductivity of the soil extract treated with the above saline solutions was measured, obtaining values of 7.4 e 81.6 mS/m, which are considered low salt stress (Brady, 2002). Soil fertilization was split in three applications at ten day intervals, providing 1.18 g/pot of N and P (NH₄H₂PO₄) and 1.40 g/pot of K (KH₂PO₄) (first application) and 1.77 g/pot of N (CO(NH₂)₂) and 1.42 g/pot of K (K₂SO₄) (second and third applications). Salinity treatments were applied 25 days after transplanting seedlings to the pots, and evaluations were 30 days after stress exposure.

Leaf sodium and potassium content

Sodium (Na) and potassium (K) contents were obtained from 0.5 g of leaf dry matter (leaf +1), as proposed by Carmo et al. (2000). Briefly, extraction was performed with 20 mL of deionized water in water bath at 100 °C for one hour. The obtained extract was centrifuged and the supernatant was analyzed in a flame photometer (Micronal, model B462) to determine nutrients contents from standard curves generated with NaCl and KCl, respectively.

Plant water status

The predawn (Ψwpred) and mid-day (Ψwmd) water potential was determined according to Scholander (1956), using a pressure chamber model M670 (Pms Instrument Co., Albany, USA). The relative water content (RWC) was determined from ten leaf disks measuring 1 cm in diameter. Initially, the discs were weighed to obtain the fresh weight (FW). Subsequently, discs were immersed in Petri dishes with deionized water for 24 hours at 20 °C in the absence of light. After this period, the discs were weighed again to obtain the turgid weight (TW). Finally, the discs were oven-dried at 65 °C for 48 hours and then the dry weight (DW) was determined. The RWC was obtained according to the formula \(\text{RWC} = [(\text{FW-DW})/(\text{TW-DW})]*100\) and expressed in % (Slavick, 1979). Assessments of water status were performed on leaf +2 and +3. Stomatal conductance was evaluated in the diagnostic leaf (+2) when fully expanded, using a diffusion porometer (Model AP4, Delta-T Devices, Burwell. Cambridge, UK).

Chlorophyll fluorescence analysis and SPAD index

The initial (Fo) and maximum fluorescence (Fm) levels and photochemical efficiency of photosystem II (Fv/Fm) of the fully expanded leaf +1 were measured in vivo after adjusting to the dark for half hour using a portable fluorometer (OS-
Leaf electrolyte leakage

Electrolyte leakage (EL) was determined from five leaf disks (leaf +2) measuring 1 cm in diameter, placed in test tubes containing 10 ml deionized water and maintained for 24 h at 25 °C in a water bath. Next, electrical conductivity of the solution was measured (L1). Thereafter, the test tubes were incubated at 90 °C for 1 h after reaching thermal equilibrium with the environment, followed by a new reading (L2) of electrical conductivity of the solution. Electrolyte leakage was calculated according to the formula E.L. (%) = (L1/L2)*100 (Lutts et al., 1996).

Plant growth measurement

Shoot and root dry/fresh weight were determined using an analytical balance. Leaf area was measured through the image analysis system Delta-T Devices LTD, using the software Delta-T Image Analysis System (Carlin and Santos, 2009).

Experimental design and statistical analysis

The experimental design was completely randomized, with ten replicates and two salt stress treatments (0 and 800 mg Na/kg soil). Data were submitted to analysis of variance (ANOVA) through the software Delta-T Image Analysis System (Carlin and Santos, 2009).

Conclusion

The results of this study show that sugarcane genotype IAC91-1099 in the initial phase of growth is sensitive to low intensity and long-term salt stress. This fact is evidenced by the reduced growth of plants under salt stress, which results from the negative impact of Na⁺ on water status, chlorophyll fluorescence and spad index. Thus, this study provides more information about the genotype IAC91-1099 in relation to its sensitivity to saline stress.

Acknowledgements

The authors thank the National Council for Scientific and Technological Development (CAPES) for providing a Master’s scholarship to the graduate student Flávio José Rodrigues Cruz for conducting this study.

References


30p, Opti-Science, USA). A SPAD meter (SPAD-502, Minolta Camera Co., Osaka, Japan) was used to estimate the SPAD values in leaves +1 following the chlorophyll fluorescence assessment.


