Influence of NaCl Salinity on Growth and Physiology of Cucumber Microshoots Grown on Rooting Medium

Sa’eed Abu-Romman and Mohammad Suwwan*

ABSTRACT

Growth and physiological responses of cucumber microshoots were studied under the influence of sodium chloride salt stress. Microshoots were grown on Murashige and Skoog (MS) rooting media containing 1.0 mg.l⁻¹ IAA. Salinity was induced by incorporating different concentrations [0.0, 25, 50, 75, and 100 mM] of sodium chloride (NaCl) to the rooting medium. Vegetative and root growth of microshoots was negatively affected by salinity. NaCl-induced salinity reduced (more negative) leaf cell sap osmotic potential. Total protein content declined significantly with increasing the concentration of NaCl, while more proline accumulated. Sodium and Cl contents were increased by salinity, while the uptake of K, Ca and P were significantly reduced.

Keywords: Cucumber, Microshoots, Rooting, Proline, Salinity.

1. INTRODUCTION

Soil salinity is a major abiotic stress in crop production worldwide (Zhu, 2001), affecting both growth and productivity (Apse et al., 1999; Kasuga et al., 1999; Xiong et al., 2002). Salinity problems are usually associated with arid and semi-arid lands (Chartzoulakis, 1992; Flowers et al., 1977). In saline environment, NaCl is usually the most prevalent salt which creates high ionic imbalance that may impair the selectivity of root membrane (Navarro et al., 1999; Yahya, 1998). According to Hasegawa et al. (1986), the general detrimental effects of salinity are due to the influence of ions on water activity of the external solution or to the direct effect of ions on the physiological and biochemical functions of the cell.

Cucumber has been classified as salt sensitive crop (Sonneveld and Voogt, 1978). The decline in cucumber vegetative growth at high salinity was expressed as reduced plant height (Chartzoulakis, 1992), stem diameter (Al Harbi, 1994), total leaf area and expansion rate (Chartzoulakis, 1994), fresh weight (Echno et al., 1997) and dry mass production (Jones et al., 1989).

In vitro culture system could be an effective alternative method to avoid soil and environmental complexities when studying plant responses to an imposed stress factor (Shibli et al., 1992). An in vitro culture provides an opportunity to control rigorously the physical environment and nutritional status and parameters which are difficult to regulate in studies with intact plants (Hasegawa et al., 1984). Consequently, homogeneity and precision of salinity treatments will be highly maintained.

Shoot apex culture has been widely used to evaluate plant physiological responses to salinity and osmotic stress in various species, including apple (Shibli et al., 2000), olive (Shibli and Al-Juboory, 2002) and tomato (Abu Khadijeh, 2002; Cano et al., 1998).

With regards to the whole plant, a similar response to salt stress could be expected in plantlets grown through in vitro shoot apex culture (Cano et al., 1998), because such explants can be considered mini-replicas of a plant with anatomical organization and ability to root and grow into a whole plant.

The objective of this study was conducted to determine the influence of in vitro-induced salinity on growth and physiology of cucumber grown on rooting media.

2. Materials and Methods

Seeds of ‘Sultan’ cucumber (Cucumis sativus L.) from Petoseeds, (CA. USA) were used in this study.

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Modified MS medium (Murashige and Skoog, 1962) was used in all micropropagation steps and in subsequent experiment. Medium was supplemented with 1% Bacto agar, 3% sucrose and 0.1 g l⁻¹ myoinositol. pH was adjusted to 5.7 prior to autoclaving at 121 °C and 15 psi for 20 minutes. Seed germination and shoot were maintained at 16 hr photoperiod using fluorescent light providing a photosynthetic photon flux density of 40 µmol m⁻² s⁻¹.

Seeds were de-coated after soaking in distilled water for 15 minutes. The decoated seeds were soaked in 70% (v/v) ethanol for 1 minute, then immersed in 1% sodium hypochlorite (using Clorox) and 6 drops of Tween-20 per 100 ml for 25 minutes. Finally, seeds were rinsed three times with autoclaved distilled water. The sterilized decoated seeds were germinated on modified MS (Murashige and Skoog, 1962) bioregulators free medium supplemented with 1% agar (Bacto) and 3% sucrose (Sigma). Cultures were maintained at 22 °C for 6 days. The cotyledonary nodes (included both cotyledons, an intact apical bud and 0.5 cm length of intact hypocotyl) from the 6 day old seedlings were dissected and inoculated on modified MS medium containing 2 mg l⁻¹ Kn for 3 weeks in order to obtain well-developed plantlets with higher number of nodal explants. Thereafter, single node stem cuttings were taken and inoculated on MS medium containing 1.0 mg l⁻¹ Kn. Two weeks later, well-developed plantlets were obtained.

Microshoots (3 cm long) were inoculated on rooting medium containing 1.0 mg l⁻¹ IAA to have in vitro culture more representative to in vivo grown plants (Shibli and Al-Juboory, 2002). Also rooting medium is more consistent to in vitro induced stress than shoot proliferation medium (Shibli et al., 1992). The medium was supplemented with different concentrations (0.0, 25, 50, 75 and 100 mM) of NaCl; Cultures were grown in 250 ml screw capped bottles containing 45 ml medium.

Data were recorded after one month for shoot length, root number and root length and their fresh and dry weights. Dry weights were determined for shoots and roots after drying samples to a constant weight at 78 °C.

Osmotic potentials were also measured on leaf samples. Leaf tissues were packed into 1 cm³ disposable syringes, and frozen at −20 °C, allowed to thaw at room temperature for 30 minutes. Cell sap was expressed from the culture samples by depressing the syringe plunger. Osmotic potential was measured on 10 µl samples of the extract sap (Shibli et al., 1992) using a Wescor 5500 Vapor Pressure Osmometer.

Free proline was extracted and colorimetrically estimated in fresh leaf samples by acid-ninhydrin method of Bates et al. (1973). Total protein content in leaves was determined according to Lowry et al. (1951), using bovine serum albumin as a standard.

For mineral composition, shoots were dried to a constant weight at 78°C. 250 mg of ground tissue were then digested with 10 ml of concentrated sulfuric acid in presence of selenium reagent mixture and heated on heating digester at 300 °C for 3 hr until clear solution was obtained. The solution was then diluted to 100 ml by adding distilled water. Sodium and potassium contents were determined by Corning 410C flame photometer. Calcium was determined using Varian Spectra AA 200 atomic absorption spectrophotometer. Phosphorus was determined according to Watanabe and Olsen (1965) wet ashing procedure and Chloride was measured by potentiometric titration (Chapman and Pratt, 1961).

Treatments were arranged in a completely randomized design (CRD). Collected data were subjected to the analysis of variance (ANOVA) and means were separated according to the least significant difference (LSD) at 0.05 level of probability using SAS program (SAS Institute, Inc., 1988).

3. RESULTS

Salt stress, caused by NaCl, resulted in a significant decline in shoot growth when microshoots were grown on rooting medium (Table 1). Each increase in NaCl concentration was accompanied with a significant reduction in shoot length. Similarly, a significant decrease was obtained with each increase in NaCl concentration, for the respective shoot fresh weights (Table 1).

Respective shoot dry weights (Table 1) decreased significantly for each increase in NaCl concentration up to the 75 Mm. Above this concentration, no significant differences were observed. The least dry weight percentages were obtained at the 75 and 100 mM concentrations. However “0 and 25 mM” as well as “25 and 50 mM NaCl” gave almost similar dry weight percentages (Table 1).
Table (1): Effect of *in vitro*-induced NaCl salinity on vegetative growth of ‘Sultan’ cucumber microshoots grown on rooting medium containing 1.0 mg.l⁻¹ IAA.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Shoot length (cm)</th>
<th>Shoot Fresh Weight (g)</th>
<th>Shoot Dry Weight (g)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.67 a(1)</td>
<td>2.64 a</td>
<td>0.1820 a</td>
<td>6.88 a</td>
</tr>
<tr>
<td>25</td>
<td>8.29 b</td>
<td>2.17 b</td>
<td>0.1391 b</td>
<td>6.41 ab</td>
</tr>
<tr>
<td>50</td>
<td>7.55 c</td>
<td>1.78 c</td>
<td>0.1125 c</td>
<td>6.33 b</td>
</tr>
<tr>
<td>75</td>
<td>6.46 d</td>
<td>1.42 d</td>
<td>0.0816 d</td>
<td>5.72 c</td>
</tr>
<tr>
<td>100</td>
<td>4.31 e</td>
<td>1.25 e</td>
<td>0.0703 d</td>
<td>5.65 c</td>
</tr>
</tbody>
</table>

(1) Means within columns followed by different letters are significantly different according to LSD (P<0.05).

Table (2): Effect of *in vitro*-induced NaCl salinity on root growth of Sultan cucumber microshoots grown on rooting medium containing 1.0 mg.l⁻¹ IAA.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Root Number</th>
<th>Root Length (cm)</th>
<th>Root Fresh Weight (g)</th>
<th>Root Dry Weight (g)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.81 a(1)</td>
<td>9.06 a</td>
<td>0.250 a</td>
<td>0.0184 a</td>
<td>7.40 a</td>
</tr>
<tr>
<td>25</td>
<td>7.93 b</td>
<td>8.18 b</td>
<td>0.227 b</td>
<td>0.0155 b</td>
<td>6.83 a</td>
</tr>
<tr>
<td>50</td>
<td>7.41 b</td>
<td>7.17 c</td>
<td>0.182 c</td>
<td>0.0111 c</td>
<td>6.09 b</td>
</tr>
<tr>
<td>75</td>
<td>6.56 c</td>
<td>6.84 c</td>
<td>0.141 d</td>
<td>0.0083 d</td>
<td>5.88 b</td>
</tr>
<tr>
<td>100</td>
<td>5.68 d</td>
<td>6.25 d</td>
<td>0.133 d</td>
<td>0.0075 d</td>
<td>5.64 b</td>
</tr>
</tbody>
</table>

(1) Means within columns followed by different letters are significantly different according to LSD (P<0.05).

Table (3): Effect of *in vitro*-induced NaCl salinity on mineral composition of ‘Sultan’ cucumber microshoots grown on rooting medium containing 1.0 mg.l⁻¹ IAA.

<table>
<thead>
<tr>
<th>NaCl (mM)</th>
<th>Concentration (mg.g⁻¹ DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
</tr>
<tr>
<td>0</td>
<td>2.63 e(1)</td>
</tr>
<tr>
<td>25</td>
<td>18.37 d</td>
</tr>
<tr>
<td>50</td>
<td>33.71 c</td>
</tr>
<tr>
<td>75</td>
<td>43.28 b</td>
</tr>
<tr>
<td>100</td>
<td>52.84 a</td>
</tr>
</tbody>
</table>

(1) For each parameter, means followed by different letters are significantly different according to LSD (P<0.05).

Root number decreased significantly by NaCl- induced salinity to similar levels at the 25 and 50 mM, but each further increase in salinity was associated with a significant decrease in root number (Table 2). The longest roots (9.06 cm) were obtained from the control. Root length declined significantly at the 25 mM, but further significant and similar reduction was observed at the 50 and 75 mM of NaCl. The 100 mM gave the shortest roots (6.25 cm).

Reduction in root fresh and dry weights was significant for each increase in NaCl concentration up to the 75 mM only. The control and the 25 mM NaCl produced the highest percent root dry weights compared to the other NaCl concentrations; similar percent dry weights were obtained for NaCl concentrations less than or equal to 50 mM (Table 2).

The 75 and 100 mM NaCl resulted in the least leaf osmotic potential among all other concentrations. However, microshoots grown in the presence of the 25 and 50 NaCl, had significantly similar leaf osmotic potentials (Figure 1).

In the absence of NaCl, microshoots had the highest protein content (Figure 2). Up to 50 mM NaCl, each increase in NaCl resulted in a significant decrease in protein content. Moreover, microshoots grown on 50 up to 100 mM NaCl produced almost similar protein contents (Figure 2). Up to the 50 mM NaCl, each increase in NaCl concentration resulted in a significant increase in proline content (Figure 3). Proline accumulation by microshoots grown on rooting medium containing either 50, 75 or 100 mM NaCl was insignificant (Figure 3).
Figure (1): Effect of \textit{in vitro}-induced NaCl salinity on leaf osmotic potential of ‘Sultan’ cucumber microshoots grown on rooting medium containing 1.0mg.l\(^{-1}\) IAA. Columns followed by different letters are significantly different according to LSD (P<0.05).

Figure (2): Effect of \textit{in vitro}-induced NaCl salinity on protein content of ‘Sultan’ cucumber microshoots grown on rooting medium containing 1.0 mg.l\(^{-1}\) IAA. Columns followed by different letters are significantly different according to LSD (P<0.05).

Figure (3): Effect of \textit{in vitro}-induced NaCl salinity on proline content of ‘Sultan’ cucumber microshoots grown on rooting medium containing 1.0mg.l\(^{-1}\) IAA. Columns followed by different letters are significantly different according to LSD (P<0.05).
Each increase in NaCl concentration was accompanied by a significant increase in Na content up to 52.8 mg.g$^{-1}$ DW at the 100 mM NaCl. Significant increase in Cl content was observed with increased NaCl up to 100 mM. The 50 and 75 mM gave significantly similar Cl contents, but the highest content was observed at the 100 mM NaCl (Table 3).

The 0 mM NaCl gave significantly highest K contents (44.5 mg.g$^{-1}$ DW). At 25 and up to 75 mM, K contents were almost the same. The least K content was found at 100 mM NaCl (Table 3). Significantly highest Ca and P contents were obtained in the microshoots in absence of NaCl. Increased salinity resulted in significant reductions in both Ca and P contents (Table 3).

4. DISCUSSION

Salinity significantly affected vegetative and root growth of cucumber microshoots grown on rooting medium (Table 1 and 2). Generally, NaCl–induced salinity decreased shoot length and fresh and dry weights (Table 1). According to Tasonev et al. (1998), reduction in growth is a typical response of nonhalophyte species to salinity. The present results (Table 1) are in line with earlier findings reported in vivo. Cucumber plant length (Chartzoulakis, 1992) and stem diameter (Al-Harbi, 1994) were adversely affected by NaCl. Reductions in shoot fresh and dry weights are also well documented (Ho and Adams, 1994; Lechno et al., 1997). Alpaslan and Gunes (2001) noticed increased leaf membrane permeability of salt-stressed cucumber plants.

Greenhouse experiments revealed growth reduction of salt-stressed cucumber plants as a result of lower photosynthetic capacity and photosynthesising area (Drew et al., 1990; Ho and Adams, 1994). Salinity-induced growth reductions were so reported for melon (Del Amor et al., 1999; Mendlinger and Pasternak, 1992; Navarro et al., 1999; Shannon and Francois, 1978; Sivritepe et al., 1999) and squash (Francois, 1985).

Root growth measures were negatively related to NaCl concentrations in rooting media (Table 2). Such reductions were reported as common responses of cucumber plants subjected to salt stress (Passam and Kakouriotos, 1994). Salinity decreased rooting percentages of in vitro grown tomato shoots (Cano et al., 1998).

Leaf osmotic potential of microshoots grown on rooting media decreased with salinity (Figure 1). In cucumber, it is reported by Chartzoulakis (1994) that leaf osmotic potential declined with salinization. Similar results were achieved in other species including blackgram plant (Ashraf, 1989) and tomato (Smith et al., 1992).

NaCl caused a marked reduction in protein content of the microshoots (Figure 2). These results are substantiated by earlier findings of Ramanjulu et al. (1999) and Tsonev et al. (1998). Reduction in protein is attributed to either decrease in protein synthesis or protein breakdown due to increased proteolytic activity or by a combination of both (Roy-Macauley et al., 1992). On the other hand, proline accumulated as a common response to salt stress (Ashraf, 1989; Borsani et al., 2001; Franco et al., 1999; Perez–Alfocea et al., 1996). Buhl and Stewart (1983) concluded that cytoplasmic proline accumulation occurs to restore the balance between the lowered osmotic potential in the vacuole compared to the cytoplasm.

Generally, microshoots on rooting medium accumulated Na and Cl in presence of NaCl stress, while K, Ca and P contents were reduced (Table 3). In the control microshoots, K was the predominant cation that was replaced by Na with increasing NaCl concentrations (Table 3). Similar earlier findings on cucumber were reported by Drew et al. (1990) and Jones et al. (1989). This situation could be explained by the antagonistic relation between K and Na (Alpaslan et al., 1999 cited by Alpaslan and Gunes, 2001).

Cucumber microshoots accumulated more CI than Na in response to NaCl–induced salinity (Table 3). Such observation was also reported by Chartzoulakis (1992) in greenhouse grown cucumber and was attributed to low Na acropetal transport potential. While microshoots Ca contents were reduced by NaCl-salinity (Table 3), Ca reduction was not clear in rooting medium. These findings are in general agreement with those of Demir et al. (1999) and Ho and Adams (1994) on cucumber. However, contradictory results were obtained in melon (Del Amor et al., 1999) and cucumber (Jones et al., 1989) where Ca contents increased with salinity stress. These discrepancies are likely due to variation in experimental conditions (in vitro vs. in vivo) and the crop used.

According to Grattan and Grieve (1999), salinity reduced P content in many plant species, which agrees with present result on cucumber microshoots. However, Mohammad et al. (1998) noticed an increase rather that decrease in tissue P concentration with increased salinity in tomato.
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