SODIUM CHLORIDE TOLERANCE OF PINEAPPLE 
(ANANAS COMOSUS L. MIRRL.) IN VITRO

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ABSTRACT

This study was conducted to investigate in vitro sodium chloride (NaCl) tolerance of pineapple and which growth parameter could be a suitable tool of screening of NaCl tolerant plants. In vitro propagated pineapple explants were cultured in MS medium containing six levels of NaCl (10, 20, 30, 40, 80 and 120 mM/l NaCl) and a control without NaCl. For root growth evaluation MS medium was further supplemented with 0.3 mg/l indolbutaric acid (IBA). The experimental units were in a completely randomized design with four replications. Both cultures were incubated for six weeks under controlled conditions. The growth parameters (explant shoot length, leaf and root numbers and shoot fresh and dry weights) and the correlation coefficients between in vitro NaCl tolerance and growth traits (shoot length and leaf and root number) were recorded. The results showed that pineapple explants growth (all growth traits except leaf number) was firstly significantly (LSD at 0.05) affected by the lower NaCl concentrations (up to 30 mM/l NaCl ) compared to control, but were not significantly affected as NaCl concentrations were further increased up to the highest one (120 mM/l NaCl). Rooting was the most NaCl sensitive parameter as root number decreased to 33% at 30 mM/l NaCl and almost stopped (only 7% of control root number) at the highest NaCl concentration (120 mM/l). However, root growth in vitro was inconsistent as different growth rates were noticed even by the control. The correlation coefficients between NaCl tolerance and some growth parameter (shoot length and leaf and root number) were highly significant (r = -0.8339**, r = -0.9799** and r = -0.8479**, respectively). It could be concluded that pineapple may be considered as moderately NaCl tolerant. Explant shoot length might be a suitable tool of in vitro screening of NaCl tolerance. However, further studies on the relation between this trait and in vivo (field) plant growth, yield and mechanism of salt tolerance is required.

Key words: Ananas comosus, pineapple explants, In vitro screening, sodium chloride tolerance, growth parameters
grown under plastic houses in newly reclaimed areas which are affected by salinity. Water and soil salinity are considered as some of the most important stress factors of agriculture, especially in arid and semi-arid areas of the world. Such a problem can be solved by growing of salt tolerant productive crops. Selection of salt tolerance lines continues to challenge plant scientists, especially those working in physiology and genetics (Flowers, 2004).

Classical pineapple breeding methods are based on crosses, back-crosses and selection (Hamed and Ali, 2007). These techniques along with the long generation cycle of pineapple, result in extremely time consuming breeding programmes. Tissue culture, which was considered an efficient method for rapid in vitro clonal propagation of pineapple long ago (Mapes, 1973 and Hosoki and Asagira, 1980) may offer potential for rapid screening and breeding of salt tolerant genotypes. Auxillary bud/shoot apex culture has been found to be an effective method for isolating salt tolerant potato genotypes from a large population within a short period of time (Naik and Widholm, 1993; Cano et al., 1989). Hamed and Ali (2007) tested the salinity tolerance of pineapple explants using different concentrations of sea water. They found that all salinity levels used, depressed their vegetative growth, if they were added directly through three subcultures. However, the greatest vegetative growth (number of leaves and rooting percentage) were obtained at the highest concentration (1000 ppm) by the gradual transfer of the explants from control to higher concentration by each sub-culture. However, the greatest vegetative growth (number of leaves and rooting percentage) were obtained at the highest concentration (1000 ppm) by the gradual transfer of the explants from control to higher concentration by each sub-culture, that is, from control sub-cultured on 500 ppm and then sub-cultured on 1000 ppm sea water. Barroso et al. (2003) tested pineapple plants which were micro-propagated for six months in a medium with 0, 12.5 and 25 mM/l sodium chloride (NaCl). The traits evaluated were plant height, plant diameter, leaf number, length and width of the leaf. There was no effect of different levels of NaCl during the micro-propagation on the traits and mean growing rates. Differences were found in the correlation coefficients between the characters and mainly, in the phenotypic variances. Their results indicated that the phenotypic variances were more adequate to evaluate the impact of in vitro selection for NaCl tolerance on traits apparently not related to salinity in pineapple plants. Hasan and Abdullah (2007) showed that the growth of pineapple under tissue culture condition was not inhibited by salt concentration below 135 mM/l NaCl. At 200 and 250 mM/l NaCl plantlets growth was significantly reduced but was not completely inhibited. Hanafi et al. (2010) evaluated pineapple growth in a field irrigated with sea water where its potassium was substituted with different amounts of sodium. They found that it was only significantly affected at later stages, if 60% of potassium was replaced by sodium.

It is clear that in vitro and field studies on pineapple salinity tolerance are rare compared to other crops. Nevertheless, in vitro screening for salinity tolerance requires an easily evaluated growth parameter as a screening tool. Moreover, a correlation between salinity tolerance mechanisms in vitro and in vivo should be realized. This study was conducted to investigate in vitro NaCl tolerance of pineapple and which growth trait could be a suitable tool of in vitro screening of NaCl tolerant pineapple genotypes.

MATERIAL AND METHODS

The study was conducted in the Plant Tissue Culture Laboratory of Leena’s Company, Kadro, Khartoum North, using explants of pineapple (Ananas comosus L) cultivar “smooth caynee”. In vitro propagated explants of almost similar sizes were cultured in MS (Murashige and Skoog, 1962) supplemented
with six sodium chloride (NaCl) levels (10, 20, 30, 40, 80 and 120 mM/l NaCl) and a control without NaCl. The medium pH was adjusted at 5.8. The medium was placed in test tubes (each of 10 ml of medium and one explant). After six weeks incubation, the vegetative growth of half of the explants was evaluated, whereas the second half was further cultured for rooting (Idris, 2002) in test tubes (one explant each) containing 10 ml of the same medium (MS + the same NaCl levels) supplemented with 0.3 mg/l indolbutaric acid (IBA). Both cultures (for evaluation of shoot and root growth) were incubated for six weeks under controlled conditions (27°C ± 1 temperature and light intensity of 3000 lux by 16 h day length). For evaluation, NaCl tolerance growth traits (explants shoot length, number of leaves, number of roots and fresh and dry weights of explants) and the correlation coefficients between tolerance and growth traits (shoot length and leaf and root number) were recorded. The experimental units were distributed in a completely randomized design (Snedecor and Cochran, 1986) with four replications and each replicate consisted of 10 explants for shoot and root growth evaluation. The data were analyzed using MSTATC package (version 3) and the least significance difference (LSD at P ≤ 0.05 or 0.01) for the separation of the means.

RESULTS AND DISCUSSION

The results showed that explants shoot length and leaf number were negatively affected as NaCl levels were increased (Table 1). The reduction of explants length was significant (LSD at 0.5) only as NaCl concentration was raised to 30 mM/l NaCl compared to control. However, no further significant differences were noticed with increased NaCl concentrations up to the highest one (120 mM/l NaCl). The leaf number was only noticeably (not significant)

<table>
<thead>
<tr>
<th>NaCl Concentration (mM/l)</th>
<th>Explant shoot length (cm)</th>
<th>Number of leaves explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>1.0</td>
<td>17.7</td>
</tr>
<tr>
<td>10</td>
<td>0.8</td>
<td>17.1</td>
</tr>
<tr>
<td>20</td>
<td>0.8</td>
<td>16.2</td>
</tr>
<tr>
<td>30</td>
<td>0.7</td>
<td>16.3</td>
</tr>
<tr>
<td>40</td>
<td>0.7</td>
<td>15.3</td>
</tr>
<tr>
<td>80</td>
<td>0.6</td>
<td>14.4</td>
</tr>
<tr>
<td>120</td>
<td>0.6</td>
<td>13.0</td>
</tr>
<tr>
<td>LSD at P ≤ 0.05</td>
<td>0.3</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 1: Effect of NaCl on shoot growth (shoot length and number of leaves) of pineapple explants in vitro

<table>
<thead>
<tr>
<th>NaCl Concentration (mM/l)</th>
<th>Explant fresh weight(mg)</th>
<th>Explant dry weight(mg)</th>
<th>Number of roots/explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>11.3</td>
<td>0.7</td>
<td>.8.1</td>
</tr>
<tr>
<td>10</td>
<td>8.0</td>
<td>0.5</td>
<td>5.9</td>
</tr>
<tr>
<td>20</td>
<td>6.2</td>
<td>0.5</td>
<td>5.6</td>
</tr>
<tr>
<td>30</td>
<td>4.7</td>
<td>0.3</td>
<td>2.7</td>
</tr>
<tr>
<td>40</td>
<td>4.3</td>
<td>0.3</td>
<td>2.1</td>
</tr>
<tr>
<td>80</td>
<td>4.3</td>
<td>0.3</td>
<td>1.8</td>
</tr>
<tr>
<td>120</td>
<td>3.0</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>LSD at P ≤ 0.05</td>
<td>0.3</td>
<td>0.1</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 2: Effect of NaCl on growth (shoot fresh and dry weights) and rooting (number of roots) of pineapple explants in vitro

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reduced as NaCl concentration was raised to 80 mM/l or higher.

As shown in Table 2, both fresh and dry weights were negatively affected with increased NaCl concentration. The reductions of both were significant (LSD at 0.05) even at the lowest NaCl concentration (10 mM/l NaCl), especially by explants fresh weight. The number of roots of explants was also significantly reduced at 20 mM/l NaCl or higher. It was almost stopped by the highest NaCl concentration (120 mM/l NaCl), where, it was only 7% of the control. Similar results were obtained by Hamed and Ali (2007) where, different sea-water concentration up to 1000 ppm reduced the growth of pineapple explants in vitro. Barroso et al. (2003) tested pineapple plants which were micro propagated for six months in a medium with 0, 12.5 and 25 mM/l NaCl. The traits evaluated were plant height, plant diameter, leaf number, length and width of the leaf. There was no effect of different levels of NaCl on the traits and the mean growing rate during the micro propagation.

Hasan and Abdullah (2007) reported that the growth of pineapple explants under tissue culture condition was not inhibited by salt concentration below 135 mM/l NaCl. At NaCl concentrations of 200 and 250 mM/l, plantlets growth was significantly reduced but was not completely inhibited.

Hanafi et al. (2010) observed a significant reduction in plant height of pineapple irrigated with different concentrations of sea water in the field, only if 60% of potassium of sea water was replaced by sodium. They suggested that growth reduction might be due to nutritional imbalance, influenced by nutrient availability, competitive uptake and transport or portioning within the plant. Maathuis and Amtmann (1999) and Rashid et al. (2004) observed that reduction in membrane stability might cause cations leakage from the roots.

The correlation coefficients (Figure 1) between salinity and the number of roots, number of leaves and shoot length were also highly significant (P<0.05 and 0.01). The root

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**Figure 1: Correlations coefficients between NaCl concentrations and *in vitro* NaCl tolerance of pineapple (as explant shoot length and leaf and root number)**

**P<0.01**
growth was the most sensitive trait to salinity. However, its sensitivity was inconsistent due to the differences in its growth rate even by the control (NaCl free medium). Nevertheless, the correlation coefficients between leaf or root number and explant shoot length were also highly significant \((r = 0.9799\) and \(r = 0.874\), respectively). Similar results were obtained by Barroso et al. (2003), where they concluded that phenotypic variances were more adequate to evaluate the impact of in vitro selection for NaCl tolerance on traits apparently not related to salinity in pineapple plants.

For in vitro screening of salinity tolerance of large numbers of genotypes, an easily evaluated growth parameter, versus yield in ex vitro screening (Naik and Widholm, 1998; Elhag et al., 2009) which is highly correlated with the mode or mechanism of salinity tolerance of glycophytes (restriction of toxic ion transport to the shoot or its translocation to the older plant parts) will be required (Dalton and Poss, 1990; Marschner, 1995; Yufdy, 2004). From the above mentioned correlations, it was clear that root growth was the most sensitive trait to salinity. However, it was not suitable as a tool for NaCl screening due to its inconsistent sensitivity in addition to its difficult assessment (easily damaged by assessment). Therefore due to the high significant correlation between explant shoot length and NaCl tolerance, between it and other growth traits and its easy evaluation, it could be considered a suitable trait for in vitro NaCl tolerance screening. However, a correlation between its in vitro and in vivo (field) growth, yield and salinity tolerance mechanism should be found.

CONCLUSION

It could be concluded that root formation was the most sensitive parameter as it was significantly affected by the lower NaCl levels (10 and 20 mM/l) and almost inhibited by NaCl levels above 120 mM/l, but its in vitro growth was inconsistent. Pineapple explants were noticeably affected by lower NaCl concentrations (below 20 mM/l NaCl) but were not further affected by higher ones (up to 120 mM/l NaCl), showing that pineapple could be considered moderately NaCl tolerant. Explant shoot length might be a suitable tool of in vitro screening of NaCl tolerance. However, further studies on relation between this trait and in vivo (field) plant growth, yield and mechanism of salt tolerance is required.

REFERENCES


