PROPAGATION AND CALLUS REGENERATION OF POTATO (SOLANUM TUBEROSUM L.) CULTIVAR ‘DESIREE’ UNDER SALT STRESS CONDITIONS

Atheel Najib Yousef1, Gharbia Hrms Danial2, Diaa Ayoub Ibrahim3, Sazan Elias Barakat4

1 College of Science, Department of Biology, University of Duhok. E-mail: atheel.yousef@uod.ac
2 College of Science, Scientific Research Center, University of Duhok / E-mail: gharbia.danial@uod.ac.
3 College of Science, Scientific Research Center, University of Duhok, diaa.ibrahim@uod.ac
4 College of Science, Scientific Research Center, University of Duhok

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ABSTRACT
Under laboratory conditions, segments of potato with single nodes were exposed to varying doses of Sodium Chloride (NaCl) (0 mM, 20 mM, 40 mM, 60 mM, 80 mM and 100 mM), using the Murashige and Skoog growth medium to assess how NaCl salt stress affects micropropagation, callus formation, and regeneration in the ‘Desiree’ potato plant cultivar, and also seeks to determine the ability of this cultivar to thrive in salt stress conditions. The data were collected after a six-week period for each salt treatment. Remarkably, a significant increase in the mass of green and dry stems and roots was observed specifically under the 40 mM NaCl treatment. Conversely, the length of shoots and branches experienced a reduction as the NaCl concentration increased. The overall impact of the NaCl treatments strongly influenced root weight. Concerning the formation (development) and revitalization of callus, segments of potato microtubers were exposed to the above-mentioned NaCl concentrations, resulting in an intermediate level of salt stress that significantly reduced callus weights as NaCl concentration increased. Furthermore, a gradual decrease in the regeneration rate was noted with increasing concentrations of the plant growth regulator BA (1 to 4 mg/l). The most profound relative regeneration rate occurred at 1 mg/l BA. Notably, the counteractive effect of salt was more apparent with higher NaCl concentrations, with exceptions at 20 mM and 40 mM. These findings propose that the ‘Desiree’ potato cultivar exhibits moderate tolerance to salt stress and indicates a capacity to endure salinity. Moreover, this valuable variety could potentially be harnessed through genetic manipulation to enhance its salt resilience.

KEYWORDS: Solanum tuberosum; Desiree; Murashige and Skoog; NaCl; Microtubers; B

1. INTRODUCTION

Potato plants (Solanum tuberosum L.) rank as the fourth most globally consumed crop, following wheat, corn, and rice. These corps serve as significant sources of fiber, minerals, vitamins, and phytochemicals, supplying sufficient energy for various life forms (organisms). Moreover, the need to enhance potato cultivation and production assumes significance to ensure sustained food availability as the world population continues to grow (1,2). Among biotic pressure factors, salinity plays an essential role that significantly impacts plant growth and productivity by disrupting essential physiological processes. The accumulation of Na+ and Cl– ions within plants can lead to highly toxic effects, causing disruption in crucial enzymatic functions (3,4). Soil salinity represents a considerable global challenge that adversely affects plant growth and productivity, contributing to economic losses in agriculture productivity, particularly in aridity and semi-arid regions worldwide. The impacted plants experience disruption due to ionic toxicity, osmotic alterations, diminished photosynthesis, and impaired nutrient transport (5,6).

Despite its inherent ability to tolerate non-biological stress factors, potato cultivation experiences a counterproductive effect, ultimately leading to a reduction in both the growth and harvest yield of potatoes (7,8). Plant tissue culture is a valuable and cost-effective method for enhancing agronomic performance and future plant advancements, such as augmenting crop productivity (9,10,11). In numerous instances, traditional breeding methods have not yielded the intended outcomes. Recently, using in vitro plant tissue culture techniques has provided a solution to important challenges related to enhancing crops for sustainable agriculture, particularly in changing climates (12). In Iraq, the main issue with low potato crop productivity is the negative impact of salinity on crop production. It is important to focus on creating resources to develop potato productivity and achieve higher levels of production (13). The aim of this research is to assess how NaCl salt stress affects micropropagation, callus formation, and regeneration in the Desiree potato plant cultivar. It also seeks to determine the ability of this cultivar to thrive in salt stress conditions.

2. MATERIALS AND METHODS
2.1. Plant Micropropagation

Auxiliary buds from in vitro ‘Desiree’ potato plant cultivar were utilized in the experiments. The samples were maintained at a temperature of 25± 2°C with a light duration of 16/8 hours, receiving 45 µMol of LED cool white light. The growth MS growth media (Murashige and Skoog, 1962) was combined with varying concentrations of NaCl (0, 20, 40, 60, 80, and 100 mM). The growth media was supplemented with added casein hydrolysate 100 mg/l, inositol 100 Mg/l, and 30g/l of sucrose. The pH of the growth medium pH was adjusted at 5.7 of using (NaOH) or (HCl), then 7g/l of agar was added to the culture for solidification and then dispensed in 250 ml Mason jars (25 ml each), The culture media was autoclaved at 121°C and 1.05 kg/cm² for twenty minutes and allowed to solidify at room temperature. Segments of plant stems, each consisting of a single node, were placed on the medium surface and covered with non-colored PVP caps secured with rubber bands. Three explants were cultured in each culture jar. The vegetative growth outcomes were observed six weeks after incubation. The fresh and dry weight of both branches and roots were measured during harvest. The dry weight was recorded after 60 hours of oven
drying at 60°C.

2.2. Callus Induction and Plant Renewal

Microcuttings obtained from in vitro cultivated potato plants were cut and placed in the culture medium. Different concentrations of NaCl (0, 20, 40, 60, 80, and 100 mM) were added to the MS growth medium in addition to 3% sucrose and 100 mg/l inositol used for callus promotion. Two-month-old callus was moved to MS medium supplemented with various amounts of BA (1, 2, 3, and 4 mg/l). Subsequently, the percentage of callus regeneration was noted after an 8-week period.

2.3. Experimental Design and Data Analysis

During the experiments, each NaCl concentration was repeated five times, and the design followed a Completely Randomized Design (CRD). To compare the results of all experiments, Duncan's multiple range test (P < 0.05) was conducted using a computerized program (SAS).

3. RESULTS AND DISCUSSION

The findings of this study provide clear evidence that the amount of NaCl increases, the growth of non-reproductive plant parts, measured in most of the experiments, decreases (Fig. 1A, Table 1) except the 20 and 40 mM NaCl.

Table 1: Impact of various NaCl concentrations in Potato plant vegetative growth (shoot system) after 6 weeks in culture on MS medium.

<table>
<thead>
<tr>
<th>NaCl Con. (mM)</th>
<th>High shoot length (cm.)</th>
<th>Shoot length average (cm.)</th>
<th>Weight of hoot fresh(g.)</th>
<th>Weight ofShoot dry(g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>8.79±0.627 A</td>
<td>4.42±0.657 A</td>
<td>0.336±0.028 A</td>
<td>0.078±0.005 A</td>
</tr>
<tr>
<td>20</td>
<td>9.73±0.791 A</td>
<td>4.83±0.820 A</td>
<td>0.402±0.052 A</td>
<td>0.086±0.013 A</td>
</tr>
<tr>
<td>40</td>
<td>10.07±1.190 A</td>
<td>4.64±0.111 A</td>
<td>0.648±0.087 A</td>
<td>0.144±0.021 A</td>
</tr>
<tr>
<td>60</td>
<td>6.10±0.208 B</td>
<td>3.41±0.101 A</td>
<td>0.268±0.031 A</td>
<td>0.070±0.010 A</td>
</tr>
<tr>
<td>80</td>
<td>5.23±0.370 B</td>
<td>3.97±0.417 A</td>
<td>0.272±0.021 A</td>
<td>0.066±0.005 A</td>
</tr>
<tr>
<td>100</td>
<td>5.86±0.244 B</td>
<td>3.90±0.247 A</td>
<td>0.290±0.041 A</td>
<td>0.072±0.008 A</td>
</tr>
</tbody>
</table>

The highest shoot length of 10.07 cm was achieved in the 40 mM NaCl treatment, followed by a length of 9.73 cm in the 20 mM NaCl treatment (Fig. 1B, Table 1). These measurements were notably higher compared to the other NaCl treatments, although not surpassing the control treatment (8.79 cm). This could be due to the plant’s adaption to the salty conditions, which has improved its ability to handle the presence of salt in its environment. (14). Furthermore, the number of new shoots varied based on the NaCl concentration treatment. As the concentration of salt (NaCl) increased progressively in the treatments, there was a consistent decrease in the average number of shoots across all NaCl treatments, in contrast to the control treatment with 0 mM NaCl. Meanwhile, there was a decline from 9.33 shoots/plant in the control treatment to 7.06 shoots/plant at the 20 mM NaCl treatment (Fig. 1 B, Table 1), but this reduction was not statistically significant.

This considerable decrease in the number of shoots was observed, dropping from 9.33 in the control treatment to 4.99, 3.73, 2.79, and 3.00 shoots/plant in the 40, 60, 80, and 100 mM NaCl salt treatments, respectively. (Fig. 1 C, Table 1). This significant decrease in the number of shoots due to salinity stress has been observed in various plant varieties. This can primarily be attributed to the effects of osmotic pressure and ion toxicity, which collectively result in reduced plant growth (15,16).

The highest average shoot lengths of 4.83 cm and 4.64 cm were observed in the 20 and 40 mM NaCl treatments, respectively. However, these measurements did not display significant differences when compared to the control treatments, which had an average of 4.42 cm, as well as with all the other treatments.

The selections align with previous research that has demonstrated the negative impact of rising salt concentrations on the growth and development of potato plants (3, 7, 17, 18, 19, 20). This suggests that the addition of NaCl to the growth medium reduces the medium’s osmotic capacity, creating salinity stress that adversely affects the growth of potato plants.

The highest fresh weight, averaging 0.648 g, was observed in the 40 mM NaCl concentration, surpassing that of the control treatment. This was followed by the 20 mM NaCl treatment, which had an average weight of 0.402 g. However, this difference was not significant compared to the control treatment, which had a weight of 0.336 g. (Fig Table 1).

The economic advantage primarily lies in the weight of fresh potatoes. The consistent decrease in weight as the NaCl dosage increases demonstrates that this trait is notably affected by high concentrations of salt. These findings are consistent with the observations of numerous researchers (19, 21, 22) who have studied the effects of increased NaCl concentrations on variables such as the fresh weight of potato shoots and roots.

The influence of salinity on shoot dry weight became evident in the MS medium enriched with 40 mM NaCl, which displayed a significant increase to 0.144 g. This was notably higher than the control medium’s dry weight of 0.078 g. and higher than the dry weights of other saline media as well.

The findings of this research align with the results of Aghaei et al. (2009), who suggested that the white potato variety Desiree shows moderate resistance to salt stress. Recent studies have illuminated a range of adaptive responses to salinity stress at the cellular, molecular, metabolic, and physiological levels (23).

Significant differences were noted in root numbers (Table 2). The data exposed that the highest root count (9.72) was achieved at 40 mM NaCl, closely followed by 9.66 at 20 mM NaCl (Fig. 1, C). However, these increases in root numbers did not show significance when compared to the control (9.40). Moreover, elevated salt concentrations led to a reduction in root numbers. Consistent findings were reported by (3, 24, 25), which observed diminished root development in potato plants subjected to salt stress conditions.
The highest average root length (12.13, 11.23, and 11.11 cm) was recorded by adding NaCl at concentrations of (80, 100, and 60) mM, respectively, into the feed medium. This differed significantly from the control treatment. Additionally, there was a slight increase in average root length at 20 and 40 mM NaCl concentrations compared to the control (Fig. 1D). The results displayed notable differences in both fresh and dry weight among the applied treatments, as indicated in Table 2. The highest fresh weight (0.342 g) was recorded at NaCl concentration of 40 mM, followed by 0.300 g at 20 mM NaCl concentration. This value was the highest of all NaCl concentrations, including the control at 0.196 g. The lowest fresh weight of 0.100 g was achieved with an 80 mM NaCl concentration. Conversely, the highest dry weight value of 0.038 g was attained at NaCl concentration of 40 mM, surpassing all other treatments, including the control. The lowest dry weight of 0.014 g was recorded at 100 mM NaCl concentration, representing the lowest value among all treatments. This observation has been consistent in numerous studies, where an increase in salinity levels has been shown to have negatively effects on plant growth and performance. This ultimately leads to a reduction in plant biomass, as reported in various studies (14,19,26,27).

Callus induction and plant renewal
As seen in Table 3, the initiation of callus was affected by NaCl concentrations. With an increase in NaCl levels, there was a distinct decrease in the callus weight.

Table 3: The impact of various NaCl concentrations in Potato microtubers callus weight after 8 weeks of cultured.

<table>
<thead>
<tr>
<th>NaCl Concentration (mM)</th>
<th>Root No./ explant</th>
<th>Average of root length (cm.)</th>
<th>The weight of root fresh (g.)</th>
<th>The Weight of root dry (g.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>9.40±0.244</td>
<td>7.266±0.734</td>
<td>0.196±0.028</td>
<td>0.022±0.002</td>
</tr>
<tr>
<td>20</td>
<td>9.66±0.708</td>
<td>8.76±0.953</td>
<td>0.300±0.056</td>
<td>0.030±0.005</td>
</tr>
<tr>
<td>40</td>
<td>9.72±0.845</td>
<td>8.39±0.485</td>
<td>0.342±0.043</td>
<td>0.038±0.004</td>
</tr>
<tr>
<td>60</td>
<td>5.80±0.827</td>
<td>11.14±0.906</td>
<td>0.148±0.026</td>
<td>0.022±0.003</td>
</tr>
<tr>
<td>80</td>
<td>4.93±0.323</td>
<td>12.13±1.057</td>
<td>0.100±0.012</td>
<td>0.016±0.002</td>
</tr>
<tr>
<td>100</td>
<td>7.13±0.826</td>
<td>11.23±1.057</td>
<td>0.112±0.020</td>
<td>0.014±0.002</td>
</tr>
</tbody>
</table>

The highest callus weight was observed in media supplied with 20 mM NaCl, recorded at 2.160 g (Fig. 1, E and F). This was followed by 1.720 g in MS media with 40 mM NaCl (Fig. 1, E and F), which significantly differed from other treatments, but the lowest callus weight of 0.748 g was noted at 100 mM NaCl (Fig. 1 H and J). Nevertheless, there were no significant differences observed among the other media containing various levels of NaCl. It can be concluded that the callus weight gradually decreased with increasing NaCl concentrations. These records are in agreement with studies conducted by (28,29,30) which suggest that increased salinity can lead to a reduction in callus growth in potato plants.). The overall outcomes indicate that high salinity can reduce plants’ ability to absorb mineral nutrients like N, Ca, P, K, Fe, and Zn ions. Additionally, the increased level of Na⁺ and Cl⁻ within plant cells can be toxic. Conversely, the high level of Na⁺ disturbs the absorption of K⁺ ions in the cells, which play a crucial role in maintaining osmotic balance and controlling the opening and closing of stomata. Furthermore, NaCl has the ability to reduce cell permeability to water, resulting in a reduction in the rate of water entering the cells (2,4,31,32).

Table 4: The impact of different concentrations of BA in callus renewal after two months of callus in MS medium.

<table>
<thead>
<tr>
<th>BA concentration (mg/Liter)</th>
<th>Regeneration percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1mg/Liter</td>
<td>66.67</td>
</tr>
<tr>
<td>2mg/Liter</td>
<td>33.33</td>
</tr>
<tr>
<td>3mg/Liter</td>
<td>46.66</td>
</tr>
<tr>
<td>4mg/Liter</td>
<td>46.67</td>
</tr>
</tbody>
</table>
REFERENCES


