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EFFECTS OF SALINITY ON GROWTH AND CHLOROPHYLL CONTENT OF *Aglaonema simplex* CULTURES

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Abstract: The effects of salinity on growth and chlorophyll content were studied in *Aglaonema simplex* cultures. The cultures were subjected to 0, 25 and 50 mM of NaCl for 28 days. The growth and total chlorophyll contents were measured every 0, 1, 2, 7, 14 and 28 days of treatment periods. NaCl treatments significantly increased the fresh and dry weights of the cultures while the plant height, root length, leaf length and leaf width were significantly decreased, especially at the latter stages of treatment periods. NaCl also caused a decrease in the chlorophyll content, especially after 2 days of experiment. The results indicate that NaCl caused oxidative stress in *A. simplex* cultures which affect their growth and chlorophyll content.

KEYWORDS: *Aglaonema simplex, Cultures, Salinity, Growth, Chlorophyll content*

Introduction

Plants are exposed to sudden daily and seasonal changes in the environment and they show a variety of developmental responses and biochemical adaptations to stress conditions. Various environmental stresses can affect plant growth, metabolisms and productivity (Smirnoff, 1998). Salinity is one of the major environmental stresses and a considerable constraint to crop production. Increased salinisation of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 22 years and up to 50% by the middle of the 21st century (Wang *et al.,* 2003). The effect of salinity stress to plant physiological aspects varies from total growth of plant, metabolic processes, structural aspects as well as photosynthesis and photosynthetic pigments. The worst effects of high salinity on plants can be observed at the whole plant level as the death of plant or decrease in its productivity. Most plants develop counter mechanisms either to prohibit salt from their cells or to tolerate the presence of salt within their cells. The earliest response of salinity stress is a reduction in the rate of leaf-surface expansion, followed by a termination of expansion as the stress intensities increase (Allakhverdiev *et al.,* 2000; Parida & Das, 2005).

A. simplex is one of the most favoured indoor plants as it grows easily with minimum care. Slow propagation by rhizome and their sensitivity to atmosphere limit the nursery production of many *Aglaonema* species. Application of micropropagation technology for *A. simplex* to be produced commercially may solve the problems of supply and quality of this plant. On the other hand, salinity stress may result in limiting or inducing the growth. Thus, a study on salinity effect may offer some understanding of how *A. simplex* reacts to salinity stress.

Materials and Methods

***Plant Materials*:** Established *in vitro* plantlets of *A. simplex* (Aziz *et al.,* 2008) were used in this study. Rhizomes were sliced from the plantlets and transferred into MS basal medium (Murashige & Skoog, 1962) supplemented with 6-benzylaminopurine (BAP), vitamin B5 and 30 g/L sucrose. The medium was solidified with 2.5 g/L gelrite agar and pH was adjusted to 5.7 – 5.8 using NaOH or HCl solution. The media were autoclaved at 121°C for 15 min. Subsequent subculture was carried out every two months and

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cultures were incubated in 12 hours (light/dark) photoperiod under cool, white fluorescent lamps at 27 ± 2 °C.

***Treatment Medium*:** Two-month-old plantlets with uniform size were treated with 0, 25 or 50 mM NaCl for 28 days in MS medium. The samples were incubated at 27 ± 2 °C, 12 hours (light/dark) photoperiod under cool, white fluorescent lamps.

Growth and Chlorophyll Content: Growth and chlorophyll content were determined at 0, 1, 2, 7, 14 and 28 days of treatment periods. At harvest, the fresh weights, leaf width and length, plant height and root length were determined. Tissue was dried to a constant weight in an oven at 80ºC to obtain dry weight. Chlorophyll content was determined according to the method of Harborne (1984). 0.15 g fresh leaf tissues were homogenised in 6 ml of 80% acetone with clean sand in mortar and pestle. The homogenate was then centrifuged at 10000 rpm for 10 min. Supernatant absorbance was read using spectrophotometer at 645 and 663 nm. Control used was 80% acetone.

***Statistical Analysis*:** Data obtained were subjected to analysis of variance (One-Way ANOVA) using Statistical Package for Social Sciences (SPSS) version 10.0. Multiple comparisons were performed using Tukey test at 0.05 significance levels.

Results and Discussion

Fresh and Dry Weights

The changes in fresh and dry weights of *A. simplex* in various levels of NaCl are shown in Figures 1 and 2. Salinity stress did not significantly affect (P>0.05) the fresh and dry weights of the plants up to 7 days of treatment periods. Slight increases were observed in fresh and dry weights after day 7. The increment was significantly lower (p<0.05) compared to control. Salinity influenced the biomass of *A. simplex* cultures similar to its effect on the mangrove, *Bruguiera parviflora* after 45 days of exposure to varying concentrations of NaCl (Parida *et al.,* 2004). Kurban, *et al.* (1999) and Khan*et et al.* (1999) also observed an increased growth of a leguminous plant, *Alhagi pseudoalhagi,* and of a halophyte plant, *Halopyrum mucronatum,* at low salinity. The increases in the fresh and dry weights might be related to the increase of water uptake by the plant from the medium, which dilutes the concentration on toxic ions in plant tissues, as reported in *Simmondsia chinensis* explants (Mills & Benzioni, 1992). The mechanism of increased water uptake is called "salt dilution by succulence" and is a common strategy in salt-adaptive species (Hagenmeyer, 1996). Lower biomass weight of cultures at higher salinity could probably be attributed to the reduced capacity for dilution that occurs when the toxic ion levels reach a point where the plant metabolism was severely affected by the ions toxicity.

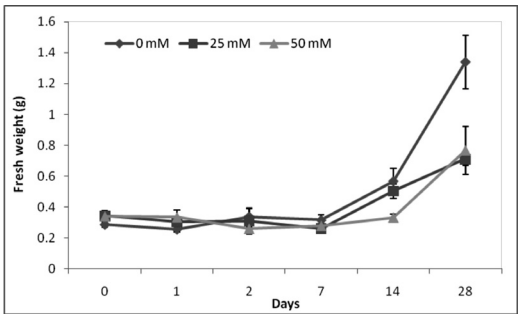

Figure 1: Changes in fresh weight of *A. simplex* cultures treated with different concentrations of NaCl. Data are means ± standard errors (n = 3).

Plant Height

A decrease in the plant height under salt stress was observed after one day of treatment period (Figure 3). However, the reduction in plant height was not significantly different (p>0.05) in cultures treated with low and high salinity. The results mimicked the research by Mohammad, Shibli, Ajouni and Nimri (1998), who reported that increasing salinity significantly reduces the height of tomato plants. This may probably be caused by high concentration of Na^+ in cells that lead to osmotic imbalance and further caused membrane disorganisation and thus would inhibit cells division and expansion (Flowers, Troke & Yeo, 1977). Furthermore, high level of Na^+ is also toxic to cell metabolism and has deleterious effect on the functioning of some of the enzymes (Niu, Bressan, Hasegawa & Pardo, 1995).

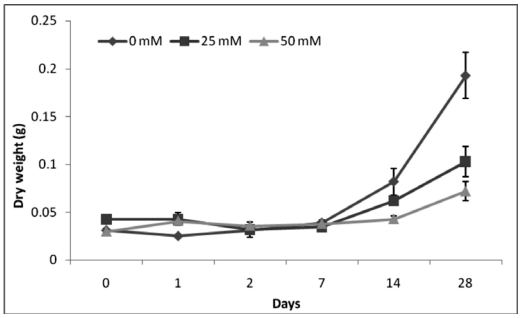

Figure 2: Changes in dry weight of *A. simplex* cultures treated with different concentrations of NaCl. Data are means ± standard errors (n = 3).

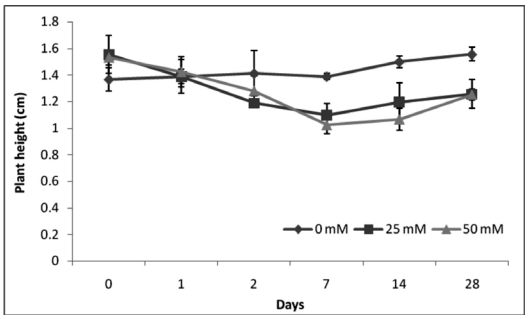

Figure 3: Changes in plant height of *A. simplex* cultures treated with different concentrations of NaCl. Data are means ± standard errors (n = 3).

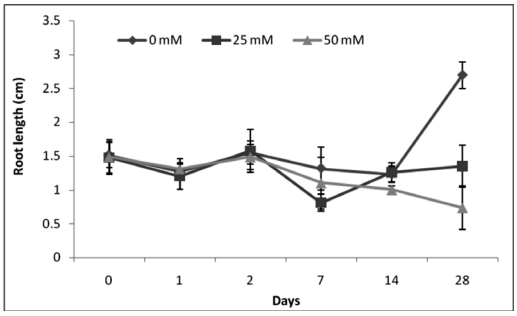

Figure 4: Changes in root length of *A. simplex* cultures treated with different concentrations of NaCl. Data are means ± standard errors (n = 3).

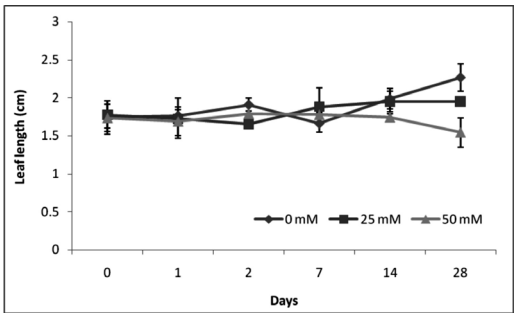

Figure 5: Changes in leaf lengths of *A. simplex* cultures treated with different concentrations of NaCl. Data are means ± standard errors (n = 3).

Root Length

Salinity stress did not affect the root length at earlier stages of treatment periods (Figure 4). However, after 14 days of treatment, the root length was significantly lower ($p<0.05$) compared to the untreated cultures. This may be due to the high sensitivity to salt of the root zone. The result is parallel with the study conducted by Jaleel *et al.* (2008a) who reported that salinity has more adverse effect on the root system over long-term exposure. NaCl induced constraint on water availability and water uptake and thus results in the reduction of root pressure-driven xylem transport rates of water and mineral nutrient (Kafkafi, 1991). Several studies have shown that, under saline conditions, Na^+ influx into root cells via Na^+ permeable transporter elevates the cytoplasmic sodium concentration and causes toxicity, resulting in a reduction of root growth (Kingsbury & Epstein, 1986).

Leaf Length and Width

The leaf length and width were not significantly affected by NaCl ($p>0.05$) throughout 7 days treatments. However, decrease in the leaf lengths and widths in the cultures treated with higher concentration of NaCl were observed after 14 days of treatment (Figures 5 and 6). The slower effect, taking days in *A. simplex* cultures, is the result of salt accumulation in leaves, leading to salt toxicity in the plant, primarily in the older leaves (i.e. salt-specific effect). This salt toxicity can result in the death of leaves and reduce the total photosynthetic leaf area as reported for *Catharanthus roseus* (Jaleel *et al.,* 2008a) and *Withannia somnifera* (Jaleel*et al.,* 2008b). As a result, there is a reduction in the supply of photosynthate to the plant, affecting the overall carbon balance necessary to sustain growth (Munns, 2002). Due to abiotic stress induced by salt, the plant tries to cope with the situation by decreasing its leaf area, hence conserving energy (Jaleel *et al*., 2008a).

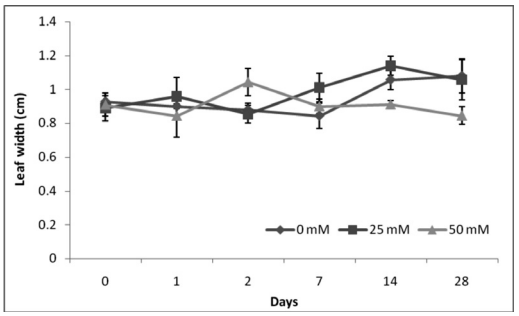

Figure 6: Changes in leaf widths of *A. simplex* cultures treated with different concentrations of NaCl. Data are means ± standard errors (n = 3).

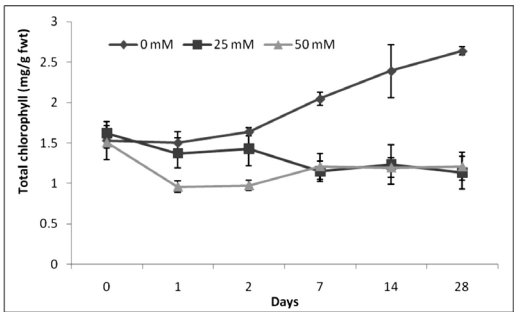

Figure 7: Changes in leaf chlorophyll content of *A. simplex* cultures treated with different concentrations of NaCl. Data are means ± standard errors (n = 3).

Effects of NaCl on the Chlorophyll Content

The changes in leaf chlorophyll content of *A. simplex* cultures within different concentrations of NaCl are shown in Figure 7. In general, NaCl treatment decreased the chlorophyll content as the treatment period progressed. However, no significant different ($p>0.05$) was observed in NaCl-treated cultures. The chlorophyll content in control cultures progressively increased ($p<0.05$) after 2 days of treatment and reached the highest content at 28 days of treatment periods. These results are in agreement with those of Khavarinejad and Mostofi (1998) for stressed tomato plants and Jaleel *et al.* (2008a) for *Catharanthus roseus*. However, a contrasting observation was reported in wheat leaves under salinity stress (Pervaiz *et al.*, 2002). Decrease in chlorophyll content in salinised plants could be attributed to increase activity of the chlorophyll-degrading enzyme, chlorophyllase (Reddy & Vora, 1986), ion accumulation (Yeo & Flowers, 1983) and decrease in carotenoids which leads to degradation of β-carotene and formation of zeaxanthins, which are apparently involved in protection against photoinhibition (Sharma & Hall, 1991). In addition, Levit (1980) reported that the reduction in leaf chlorophyll content has been attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex. It is also attributed to the interference of salt ions with the de novo synthesis of proteins, the structural component of chlorophyll, rather than the breakdown of chlorophyll (Jaleel *et al.*, 2008a). The loss of chlorophyll is often considered as a marker of a cellular component of salt stress (Singh & Dubey, 1995). Thus, these results support the hypothesis that leaves of *A. simplex* cultures were stressed when the plants were grown in a salty medium.

Conclusion

The results of this study indicated that salt stress would reduce the *A. simplex* cultures fresh and dry weights as well as decrease the stem height and root length. NaCl treatments did not significantly affect leaf lengths and widths whereas chlorophyll contents were significantly lower in treated cultures compared to the control plant, especially at longer treatment periods. Further investigations are needed to identify the suitability of this plant to be cultivated in varying salinity regimes.

Acknowlegments

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