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Effect of NaCl Stress on Pineapple Plant (*Ananas comosus* Merr. (L.) cv. Del Monte) *In Vitro*

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ABSTRACT: The present study was carried out at Plant Tissue Culture Laboratory, during the period from (15/10/2011 - 15/11/2012) to investigate effect of different sodium chloride (NaCl) concentrations on vegetative and root growth characteristics of pineapple plant via *in vitro* culture. The results showed that MS medium supplemented with 1.5 $mg.l^{-1}$ kinetin for shoot multiplication gave 360 shoots after 24 weeks from culture (three re-cultures). Also, the results showed that MS medium without NaCl treatment of shoot multiplication stage gave high significantly percentage of survival explants reached 85%. While, the MS medium supplemented with 2% NaCl gave low significantly percentage of survival explants reached 15%. The vegetative and root growth characteristics decreased when the NaCl concentration were increased in multiplication and rooting stages. The MS medium without NaCl treatment gave highest vegetative and root growth characteristics reached 12.42 cm (leaf length), 5.4 (leaves), 11.6 (shoots), 90% (rooting percentage), 4.00 cm (root length) and 7.6 (roots). But, the MS medium supplemented with 2% NaCl gave low values in there characteristics reached 1.08 cm, 3.8 leaves, 1.4 shoots, 20% rooting percentage, 1.24 cm root length and 1.4 roots. Also, the results showed that plantlets produced from MS medium supplemented with NaCl at 0.0% and 0.5% concentrations were successfully acclimatized (100%) after eight weeks from rooting stage. While, the weak plantlets of other treatments (1.5 and 2.0%) NaCl were failed in growth and acclimatization. Conclusion from present study that pineapple plantlets were low of salt tolerance and the possibility of planting of pineapple plants in Basrah government, southern Iraq.

***Keywords*:** In vitro, Sodium chloride, Kinetin, Naphthalene acetic acid, Shoot multiplication

INTRODUCTION

The pineapple plant (*Ananas comosus* Merr. (L.)) belongs to bromeliad family (Bromeliaceae), which contains 50 genera and about 2500 known species (Duval *et al*., 2003). It is a tropical plant and grows best in a moderately warm climate (16-33 °C) with low, but regular rainfall (Py *et al*., 1987). The pineapple fruit is important due to its high sugar content and attractive flavor; additionally it contains vitamins A and C (Escalona *et al*., 1999). At its apex, the fruit bears a compressed, leafy shoot called a crown (Evans *et al*., 2002). Pineapple plant is normally propagated by vegetative, means using crown, suckers and slips (Py *et al*., 1987). A large number of reports were recommended for *in vitro* shoot formation of pineapple methods (Zee and Mune Kala, 1992; Gangopadhyay *et al*., 2005; Hamad and Taha, 2008).

Salinity is considered one of the major environmental stress which adversely affect the productivity of agriculture crops. Salinity concentrations that restrict plant growth vary widely among species and plants have adapted to a wide range of saline environments. The issue of salt tolerance is expected to become more serious as human population growth in the tropics begins to compete for finite water resources (Shannon, 1992). Salt tolerance in tropical species has not been studied as extensively as in species of temperate regions. There are a number of excellent reviews concerning salt tolerance physiology in plant that probably have general applicability to tropical crops (Flowers *et al*., 1977; Cheeseman, 1988; Furr and Ream, 1968; Yeo and Flowers, 1989; Lauchli and Epstein, 1990; Shannon, 1992). Saline irrigation water causes

decrease in crop yield, length of shoot, stem and root, leaf number, fresh and dry weight and chlorophyll (Munnus and Termaat, 1986; Rhoades *et al*., 1992). Some studies were reported range of response of pineapple plant (*Ananas comosus* Merr. (L.)) to NaCl stress (Shannon, 1992; Barrose *et al*., 2003; Hamed and Ali, 2007; Hasan and Abdullah, 2007)

Because of pineapple is a rather propagating new crop by tissue culture technique in south of Iraq especially in the newly reclaimed land, and the Iraqi farmers suffer from irrigation water and soil saline problems especially south of Iraq. This present study carried out to examine pineapple plant response to NaCl stress *in vitro*.

MATERIALS AND METHODS

The experiment was carried out at the Plant Tissue Culture Laboratories, Dept. of Horticulture and Landscape Design, Agriculture college, Basrah University, Basrah, Iraq.

Source of the plant materials

Auxiliary buds with part of leaf base (0.5 cm length) of pineapple plant (*Ananas comosus* Merr. (L.) cv. DelMonte was obtained from a healthy and well established plants growing in a growth chamber. Axillary buds, were then kept in anti-oxidant solution containing 100 $mg.l^{-1}$ ascorbic acid and 150 $mg.l^{-1}$ citric acid for 24 hours to avoid phenolic compounds exudation during explants culturing. The axillary buds were then rinsed with sterile distilled water for 3 times and surface sterilized with 20% commercial chlorax solution containing 1.05% sodium hypochlorite, and a drop of tween 20 for 15 minutes. The axillary buds were then rinsed in sterile distilled water for 3 times.

Axillary shoot induction

Using full strength MS (Murashige and Skoog, 1962) basal medium supplied with kinetin at 1.5 $mg.l^{-1}$. The pH of the media were adjusted to 5.7 with 0.1 N NaOH or HCl after adding 6 % agar , and before autoclaving at 1.04 $Kg.cm^{-2}$ for 15 minutes. All media were dispensed in culture tubes containing 15 ml medium cultures. Axillary buds explants were cultured on these medium and incubated at a temperature of 27 ± 2 ˚C and light intensity under 1000 Lux light intensity provided by white fluorescent lamps for 16 hrs. The explants re-cultured to a fresh medium after eight weeks intervals. The number of produced axillary shoots was recorded after 24 weeks from culture (Plate1, A).

Effect of NaCl treatment on axillary shoot induction

The produced axillary shoots were cultured on full strength MS basal medium supplied with kinetin at 1.5 $mg.l^{-1}$. Then, NaCl at the following concentration was added: 0.0, 0.5, 1.5 and 2.0% (Plate1, B). The replication was five fold. The culture jars incubated in a growth chamber on the same conditions of light and temperature as referred to above. Vegetative characteristics of the axillary shoots were determined including: Survival percentage of pineapple shoots, Leaf length, leaf number per axillary shoot and Number of axillary shoots/explants) after eight weeks from culture.

Effect of NaCl treatment on induction of rooting

The newly formed axillary shoots obtained in the previous step were separated and transferred to a rooting medium consisting of half strength MS medium supplemented with 0.2 $mg.l^{-1}$ NAA. To this medium, NaCl at same concentrations (0.0, 0.5, 1.5 and 2.0%) was added (Plate1, F). The cultures incubated in a growth chamber on the same conditions as referred to above. The rooted shoots were obtained within eight weeks of culture on this medium. The following measurements were determined on the rooted shoots: rooting percentage, root length and number of roots per shoot.

Plantlet acclimatization

The process of acclimatization was carried out on plantlets, 8-12 cm in length, with an average of 6-8 leaves and having a good root system. Plants were removed from the culture jars and washed with sterilized water to clean the root system from the remains of the culture medium. The plantlets were then placed in glass flasks containing half strength MS medium and distilled water ensuring the submergence of the root system.

The glass flasks were then closed with thin aluminum foil and placed in a growth chamber for 24 hours. Then, the plantlets were planted in an autoclaved soil mix containing peat moss and covered with a glass tube. The acclimated plantlets were watered once a week with half strength MS salts, and distilled water was added to the pots as required. The plantlets were misted regularly with distilled water and the inner surface of the glass cover to achieve optimum humidity to prevent wilting of the plantlets. The plantlets of acclimation continued for 12 weeks and the rate of survival for different treatments was recorded.

Statistical design and analysis

Completely randomized design was used with five replicates. The data were subjected to the analysis of variance and mean values were compared using revised LSD at 5% (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

Figure 1, A showed that full MS medium supplemented with 1.5 mg.l-1 kinetin gave 20-30 shoots/explant after eight weeks from culture. The total number of shoots axilllary became 360 shoots after third re-culture (24 weeks) from shoot multiplication stage.

These results are supported by other reports on proliferation of pineapple plant by culturing in MS medium supplemented with kinetin (Wee, 1979; De Wald, 1988; Kiss *et al*., 1995, Khan *et al*., 2004).

Effect of NaCl treatment on shoots multiplication

The results showed that MS medium without NaCl (control treatment) gave high significantly increased to survival percentage of explants in multiplication stage, that reached 85% after eight weeks from culturing (Fig.2). But, those survival percentages of explants were significantly reduced when NaCl concentrations were increased (Figure 2). The MS medium supplemented with 2% NaCl gave high significantly decreased to survival percentage of explants reached 15%. The survival percentage of explants reduced to approximately half when these explants cultured in MS medium supplemented with 0.5% NaCl (47.5%), comparison with MS medium without NaCl which reached 85%(Figure 2). This similar reduction continued with 1.5% NaCl reached 20%. This concentration of NaCl (1.5%) did not significantly different with 2% (Figure 2). Hamed and Ali (2007) accepted with present results about effect of sea water salinity of pineapple explant survival via *in vitro*.

Table.1 showed that MS medium without NaCl treatment gave high values in vegetative characteristics: 12.42 cm (leaf length), 5.40 shoots (number of leaves/shoot) and 11.60 shoots (number of induction shoots/explant). This treatment (control) was significantly difference with all treatment in these vegetative characteristics except 0.5% NaCl treatment, which it's not significantly difference with control treatment in number of leaves/shoot (Table.1). That salinity stress had an immediate affect on cell divisions, leaf expansion and leaves number of plants (Parida and Das, 2005). These results accepted with other studies on effect of NaCl on pineapple shoot multiplication by *in vitro* culture (Medeiros *et al*., 2001; Barroso *et al*., 2003; Hasan and Abdullah, 2007)

Effect of NaCl treatment on rooting shoots

The results in Table.2 showed that effect of different concentrations of NaCl on root characteristics. These characteristics (rooting percentage, length of root and number of formation roots) were high significantly values, when the shoots cultured in half strength of MS medium without NaCl for rooting comparison with other treatments, there reached 90%, 4.00 cm and 7.6 roots, respectively. While, the half strength MS medium supplemented 2% NaCl gave significantly lowest in rooting percentage, length of root and number of formation roots reached 20%, 1.24 cm and 1.4 roots, respectively. Similar salinity results found by Hamed and Ali (2007) when studied effect of different concentrations of sea water on growth parameters of pineapple roots *in vitro*. Also, the results reported on *in vitro* selection of olive *Olea eauropea* L. (Fayek *et al*., 2005).

Generally, the vegetative and root characteristics were gradient increase with reduction of NaCl concentration when it was added to MS medium for shoot multiplication and rooting stages (Tables 1 and 2) and (Plate.1, C, D, E, F and G).

Plate.1 showed that plantlets produced from MS medium supplemented with NaCl at 0.0% and 0.5% concentrations were successfully acclimatized (100%) after eight weeks from culture (Plate.1, H1, H2 and I). While, the weak plantlets of two other treatments (1.5 and 2.0%) NaCl were failed in growth and acclimatization. This result was agreed with other studies about salinity effect on acclimatization of pineapple plantlets via *in vitro* (Hamed and Ali, 2007; Hasan and Abdullah, 2007). Chloride is the one of the active osmotic materials in cell vacuoles and when external osmotic pressure solutions increases than osmotic pressure of plant cells, a disorder osmotic regulation by plant cells accrues and high levels of sodium and chloride have a direct toxic effects on membrane and enzymatic systems (Shannon, 1992; Kafi and Mahdavi Damghani, 2000).

Table 1. Effect of NaCl on vegetative characteristics of pineapple shoots after eight weeks from *in vitro* culture (Shoot multiplication stage)

NaCl (%)	Leaf length (cm)	Leaf number/shoot	No. of formation shoots
0.0	12.42	5.40	11.60
0.5	7.18	5.20	6.80
1.5	1.60	4.00	3.60
2.0	1.08	3.80	1.40
R-LSD (0.05)	0.728	0.961	0.933

Table 2. Effect of NaCl treatment on root characteristics of pineapple shoots after eight weeks from *in vitro* culture (Shoot rooting stage)

NaCl (%)	Rooting percentage	Root length (cm)	No. of formation roots
0.0	90.0%	4.00	7.6
0.5	62.5%	2.82	4.6
1.5	42.5%	1.62	3.0
2.0	20.0%	1.24	1.4
R-LSD (0.05)	9.721	0.187	0.798

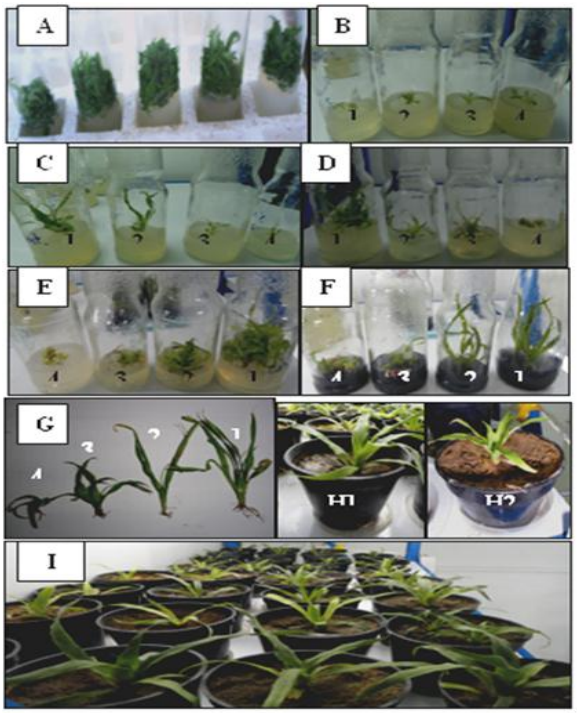

Figure 1. Effect of NaCl concentration on pineapple plants (*Ananas comosus* (L.) Merr. for salinity

A. Shoots proliferation from culturing axillary buds in MS medium supplemented with 1.5 mg.l^{-1} kinetin after eight weeks from culture (shoot multiplication stage).
B. Pineapple shoots cultured in MS medium supplemented with different concentrations of NaCl in multiplication stage.
C. Pineapple shoots cultured in MS medium supplemented with different concentrations of NaCl in multiplication stage after four weeks from culture.
D. Pineapple shoots cultured in MS medium supplemented with different concentrations of NaCl in multiplication stage after six weeks from culture.
E. Pineapple shoots cultured in MS medium supplemented with different concentrations of NaCl in multiplication stage after eight weeks from culture.
F and G. Shoot rooting in half strength MS supplemented with 0.2 mg.l-1 NAA and different concentrations of NaCl (Rooting stage).
H. Survival plantlets of pineapple produced from without NaCl treatment (H1), at 0.5% NaCl concentration (H2) after 10 weeks from culture.
I. Acclimatized plantlets after 12 weeks from culture.

1:Without NaCl treatment , 2: 0.5% NaCl treatment, 3: 1.5% NaCl treatment, 4: 2.0% NaCl treatment.

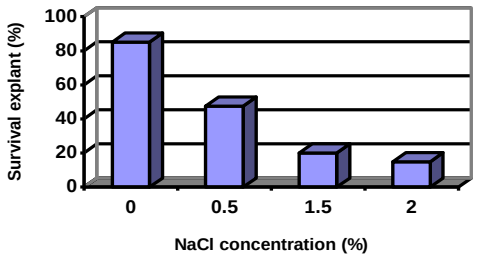

Figure 2. Effect of NaCl treatment on survival explant of pineapple plant via *in vitro* culture after eight weeks from culture.

CONCLUSION

The results of present study, that growth of pineapple cultures were not affected under low concentration of NaCl (0.5%) and its gave survival plantlets (100%). Also, pineapple plantlets were low of salt tolerance and the possibility of planting of pineapple plants in Basrah government, Southern Iraq.

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