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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

INTRODUCTION

Pineapple (*Ananas comosus* L.) of the family Bromeliaceae is an important fruit crop cultivated in tropical and subtropical regions of the world. It is a perennial monocot having a terminal inflorescence and fruit. The fruit is a good source for vitamins A, B and C, calcium, phosphorus and iron. The dry weight of the fruit contains 75-83% sugars and 7-9% citric acid (Flath, 1980). Its world production areas and yields were increased progressively between 2002 and 2007, where Thailand, Philippines, Brazil and China are the main producers with 50% of the world total production (FAO, 2009). In Sudan the production areas of pineapple were concentrated in the southern part of the country (Abdalla and Pehu, 1987). FAO statistics (2009) showed that the production and yield of pineapple increased by one percent from 2003 to 2007. However, it can be a promising crop in the central and northern parts of the country. It can also be

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, 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 (27^0C ± 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 1: Effect of NaCl on shoot growth (shoot length and number of leaves) of pineapple explants *in vitro*

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

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

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

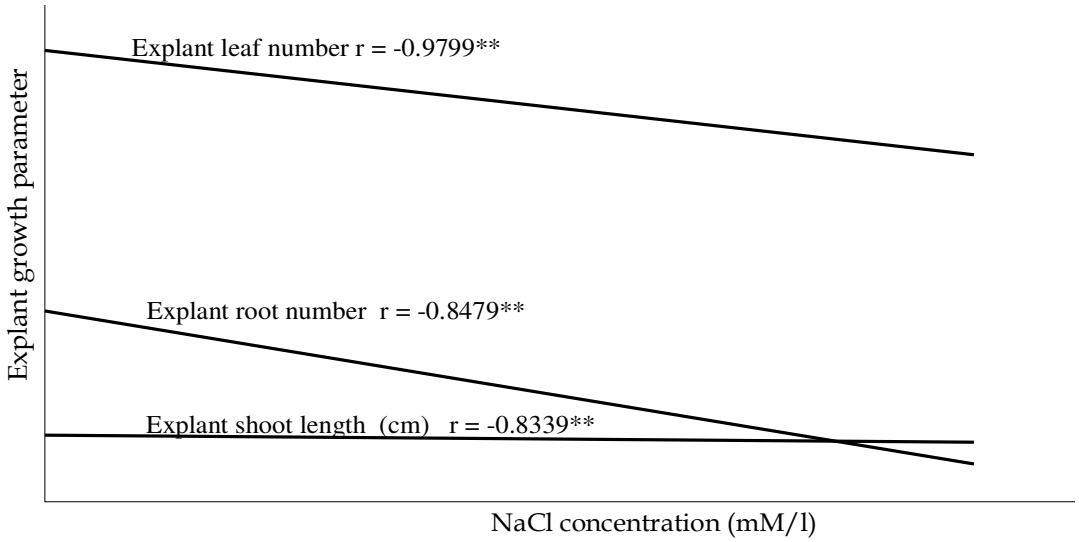

Figure 1: Correlations coefficients between NaCl concentrations and *in vitro* NaCl tolerance of pineapple (as explant shoot length and leaf and root number)

** P<0.01

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

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* or 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

Abdalla, A. and Pehu, E. 1987. Handbook on fruit production in the Sudan. FAO project SCP/SUD/030/FIN.

Barroso, P. A. V., Moura, G. E. D. D., Brito, L. K. F., Martins, C. P., Macedo, C. E. C., Lopes, D. B. and Alloufa, M. 2003. Effects of *in vitro* culture in the presence of NaCl in pineapple plants during the acclimatization phase. Review of Brazilian Engneer and Agricultural Science Ambient, 7 (3): 473 - 477.

Cano, E. A., Perez-Alfocea, F., Moreno, V., Caro, M. and Bolarin, M. C. 1998. Evaluation of salt tolerance in cultivated and wild tomato species through *in vitro* shoot apex culture. *Plant Cell Tissue and Organ Culture, 53: 19 - 26.*

Dalton, F. N. and Poss, J. A. 1990. Water transport and salt loading. A unified concept of plant salinity. *Acta Horticulturae, 278: 187 - 193.*

Elhag, A. Z., Mix-Wagner, G., and Horst, W. 2009. Difference among *Solanum* species in sodium chloride tolerance. *Sudan University of Science and Technology Journal of Science and Technology, 10 (1): 111 – 123.*

FAOSTAT. 2009. Cited from http// www.fao.org. On 11/7/2009.

Flath, R. A. 1980. Pineapple. In: Tropical and Subtropical fruits. Nagy and Shaw eds. Westport, Connecticut USA, p. 157 - 183.

Flowers, T. J. 2004. Genetics of plant mineral nutrition: Improving crop salt tolerance. *Journal of Experimental Botany, 55: 307 - 319*.

Hamed, A. M. and Ali E. A. M. 2007. Effect of different sea water concentrations on growth parameters of pineapple (*Ananas comosus*) *in vitro* and *in vivo*. *Journal of Applied Sciences Research, 3 (8): 713 - 722*.

Hanafi, M. M., Shadullahi, S. M., Niazuddin, M., Abd Aziz, Z. and Mohammud, C. H. 2010. Potential use of sea water for pineapple production in BRIS soil, International *Journal of Agricultural Biology, 12 (3): 396 - 400*.

Hasan, S. M. Z. and Abdullah, N. S. 2007. Effect of salinity on growth , proline accumilation and malate content of pineapple (*Ananas comosus* L. Merill. under tissue culture condition. *Malaysia Applied Biology, 36 (2): 57 – 63*.

Hosoki, T. and Asagira, T. 1980. *In vitro* propagation of bromeliads in liquid culture. *Horticulture Science, 15: 603 - 604*.

Idris, T. I. M. 2002. *Ex vitro* growth enhancement of micro-propagated pineapple's propagules. Ph.D Thesis, College of Agricultural Studies. Sudan University of Science and Technology, Khartoum North, Sudan.

Maathuis, F. J. M. and Amtmann, A. 1999. K^+ nutrition Na^+ toxcicity: the basis of K^+/Na^+ ratios. *Annals of Botany, 84: 123 - 133*.

Mapes, M. O. 1973. Tissue culture of bromeliads. Proceeding of International Plant Proagation Society, 23: 47 - 55.

Marschner, H. 1995. Mineral Nutrition of Higher Plants, 2nd edition,. Academic Press, New York, USA, p. 405 – 417.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia. Plantarium., 15: 473 - 497*.

Naik, P. S. and Widholm, J. M. 1993. Comparison of tissue culture and whole plant responses to salinity in potato. *Plant Cell Tissue and Organ Culture, 33 (3): 273 - 280*.

Rashid, P., Karmoker, J. L., Chakrabortty, S. and Sarker, B. C. 2004.The effect of salinity on ion accumulation and anatomical attributes in mungbean (*Phaseolus radiatus* L. cv. BARI-3) seedlings. *International Journal of Agricultural Biology, 6: 495 – 498*.

Snedecor, G. M. and Cochran,W. G. 1986. Statistical Methods. 9th ed.; The Iowa state Univ., Press. Amer. Iowa, U.S.A.

Yufdy, M. P. 2004. Utilization of seawater for pineapple cultivation. Ph.D. Thesis, University Putra Malaysia, UPM Serdang, Selangor, Malaysia.