The 2nd Faculty Annual Research Session
Faculty of Applied Science
University of Vavuniya, Sri Lanka
September 15, 2021

Influence of salinity on the morphogenic response of hypocotyl explants of tomato (*Lycopersicon esculentum* Mill.)

Shiyamala, B.
Department of Crop Science
Eastern University of Sri Lanka
dharshaniro28@gmail.com

Seran, T.H.
Department of Crop Science
Eastern University of Sri Lanka
thayaminis@esn.ac.lk

Upendri, H.F.L.
Department of Crop Science
Eastern University of Sri Lanka
lakmaupendri19931109@gmail.com

ABSTRACT

This experiment assessed the *in vitro* response of hypocotyl explants of tomato variety (KC-1) to salinity stress. Hypocotyl explants were taken from *in vitro* grown 12 days old seedlings used as explants in this experiment. The different portions of hypocotyl explants from *in vitro* grown seedlings and inoculated on Murashige and Skoog media fortified with 1.5 mg/L 6-Benzylaminopurine, 0.2 mg/L 1-Naphthaleneacetic acid and different concentrations of NaCl salt. It was observed that the morphology of the cultured hypocotyl was significantly different ($p<0.05$) from the salt and control culture media. Moreover, the results revealed that in hypocotyl explants, the different portions exhibited different responses to the salinity. The top portion of hypocotyl explants produced micro shoots directly, while the bottom portion of hypocotyl explants formed callus from the cultured explants. Four weeks after the establishment of culture, the fresh weight and callus colour was recorded, and it was compared with the salt-free media, which showed a significant difference in each portion of explant. When the salinity concentration increased, there was a significant decline in the *in vitro* response up to 4 weeks of culture. The top portion of hypocotyl produced shoots from the 35 mM salt media but with distinct necrotic patches. The fresh weight of callus was higher in the 15 mM salt media than in the control media and tended to produce shoots after transferring into a salt-free media.

Keywords: Hypocotyl explant, *In vitro* propagation, Salinity stress. .

INTRODUCTION

Tomato belongs to the family Solanaceae, and it is commercially cultivated as an important vegetable crop. It is a rich source of minerals like iron, phosphorus, vitamins A and C, and dietary fibres. It contains lycopene and beta-carotene pigments and is also cholesterol-free (Rao and Agarwal, 2000). It can be preserved in products like sauce, ketchup, chutney, soup and paste etc. Also, it is known as productive as well as protective food. Plant regeneration of tomato has been successfully achieved from different explant sources and incorporating different plant growth regulators into the media. Previous works confirmed that the hypocotyl explant could be used as a successful explant source to obtain callus (Chaudhry et al., 2004; 2010; Osman et al., 2010) and direct shoots (Abu-El-Heba et al., 2008; Rashid and Bal, 2010) for plant regeneration. Further, Shiyamala (2017) observed shoot regeneration from cultured hypocotyl segments of tomato in the Murashige and Skoog (MS) media incorporated with 1.5 mg/L 6-Benzylaminopurine (BAP) and 0.2 mg/L 1-Naphthaleneacetic acid (NAA).

Salinity is the most important abiotic factor that limits crop productivity by affecting the growth of plants (Bray et al., 2000). Salt stress impacts different processes of plant growth such as germination, dry weight of shoot and root and reduction of water potential (Parida and Das, 2005; de la Peña and Hughes, 2007). Salinity alters plant morphology, physiology and biochemistry of plant processes and ultimately reduces the yield and productivity of crops (Zhang et al., 2004; Amini and Ehsanpour, 2006; Aazami et al., 2010). Selection of salt-tolerant variants by using different screening techniques recorded to be difficult and time-consuming. *in vitro*, culture techniques are currently used for screening different salt-tolerant varieties as those cultivated plants perform their capacity to withstand the stress at different stages of the development process (Tewary et al., 2000; Zaki et al., 2016). Therefore, this study was executed to develop tomato (*Lycopersicon esculentum*) cell lines for salt tolerance by the influence of NaCl through the organogenesis of tomato.

METHODOLOGY

This experiment was conducted to study the effect of NaCl salt on *in vitro* shoot regeneration of tomatoes at the Tissue Culture Laboratory, the Eastern University of Sri Lanka, in 2017. This experiment was laid out in complete randomized design—mature seeds of tomato cv. The KC-1 was obtained from the Horticultural Crops Research and Development Institute, Department of Agriculture, Gannoruwa, Sri Lanka and used as a source of explants throughout this experiment. Surface sterilization of tomato seeds was done by spraying with 70% ethanol for 3 min followed by 5.23% sodium hypochlorite (CloroxTM) at 20% (v/v) with two drops of Tween-20 for 20 min. Then seeds were subjected to wash with sterilized distilled water four times until removing the chemicals. Seeds were then kept in a sterilized Whatman no 1 paper for germination. *in vitro* 12 days old seedlings were used to excise the hypocotyl explants for the regeneration process. MS media (1962) fortified with different concentrations of plant growth regulators, 30 g/L of sucrose and 0.8% w/v agar was used, and the pH of the media was adjusted at 5.8. Media containing culture vessels were then autoclaved at 15

(a) (b)

Figure 1: Shoot formation from the hypocotyl (near to cotyledonary node portions) explants cultured on different media (a) Control media (0 mM NaCl) after two weeks of culture (b) 15 mM media after three weeks of culture

psi at 121 °C temperature for 20 minutes. Four different NaCl solutions viz., 15 mM, 25 mM, 35 mM and 45 mM with control (0 mM), were prepared. The hypocotyl (1.0 cm long) explants were excised in three different portions, such as top portion (near to cotyledonary node), a middle portion and bottom portion (near to root base) from *in vitro* germinated seeds under aseptic conditions. They were then inoculated horizontally, half embedded in MS media fortified with 1.5 mg/L BAP and 0.2 mg/1 NAA containing different concentrations of NaCl (0, 15, 25, 35 and 45 mM) for the shoot regeneration from hypocotyl explants. The cultured explants containing culture vessels were incubated at 25 $\pm$ 0.5 °C, under fluorescent light for 16 hours photoperiod with a light intensity of 2000 lux, and 70% humidity was maintained.

After four weeks of culture in different concentrations of NaCl, characteristics such as the colour of callus, fresh weights of callus and morphology of callus were recorded. The growth response of callus on media with NaCl as compared to the control media. After four weeks, proliferated callus from different media was collected, and fresh callus weight (FCW) was determined. The growth and survival rates of the salt-tolerant cell line of tomato hypocotyl explants were determined. Under the different concentrations of salinity media, the survival rates of explants were observed, and data were collected. The collected data were subjected to analysis of variance (ANOVA) using the general linear model procedure of SAS ver9.1.3 statistical package. The mean comparisons between treatments were tested using Tukey's HSD (honestly significant difference) test at a 5% significant level.

RESULTS AND DISCUSSION

After four weeks of culture, the *in vitro* response of hypocotyl explants cultured on salinity stressed media was taken. The results revealed that salinity stress caused a significant decrease in the cultured explants of tomato (KC-1). After four weeks of culture, some top portions of hypocotyl explants produced micro shoots directly on the edge of the hypocotyls while the bottom portion of hypocotyl explants produced green calli on the surface of the cultured explants. Moreover, when the NaCl concentration in the media increased, the shoot formation was reduced in the top portion of hypocotyl, while the callus formation was also decreased in the bottom portion of hypocotyl explants. These results agreed with the findings of Mercado et al. (2000), who noticed suppressed shoot regeneration in the presence of NaCl in the media. Moreover, Mercado et al. (2000) and Aazami et al. (2010) found that increasing salinity leads to a decline in callus growth in tomatoes when selecting lines for salinity tolerance. Also, salinity creates potential water gradient, dehydrated cells, reduced callus fresh weight (Al-Khayri, 2002; Lokhande et al., 2010), and declining callus growth rate (Ashraf and Ahmad, 2000; Ehsanpour and Fatahian, 2003; Lutts et al., 2004). As NaCl levels increased in the media, it was observed that the survival rate of callus decreased.

In general, the cell growth rate was declined with all NaCl treatments. At 0 mM NaCl (non-stressed media) concentration, the explants produced micro shoots than in the other media (Figure 1a). There was a significant influence on callus morphology with different concentrations of NaCl compared with the control (0 mM NaCl). When the concentration of NaCl increased more than 35 mM, a small cluster of cells survived on the culture media, and subsequently, callus masses appeared necrotic. Comparatively, salt-tolerant callus was greenish and more compact without necrotic signs. Gupta et al. (2014) observed the callus development with NaCl and compact calli of yellowish-green colour obtained from cultured Stevia leaves. In the 45 mM salinity media, friable callus was turned from white, green to yellow, then brown after three weeks of culture. When the cultures were subjected to higher NaCl levels, toxicity symptoms appeared as reduced size, marginal necrosis, and reduced fresh weight of explant and ultimately explant necrosis. Badawy et al. (2008) also observed a reduction

Table 1: Effect of salt stress on the fresh weight of hypocotyl explants after four weeks of culture

Salt treatments (mM)	Fresh weight of callus (mg)
0 (Control)	198.34 ± 3.0 ^{a,b}
15	261.94 ± 5.0 ^{a}
25	177.59 ± 2.7 ^{a,b}
35	169.91 ± 3.4 ^{a,b}
45	151.45 ± 4.8 ^{b}

Results based on the availability of surviving explants cultured in the media. Values represent means ± standard error of the replicates. Means followed by the same letter in each row are not significantly different according to Tukey's HSD test at 5% significant level.

Table 2: Effect of different NaCl levels (0-45 mM) on callus morphology of tomato (KC-1) at four weeks of culture

Salt treatments	Hypocotyl portions	Morphological responses
0 mM (control)	Top	Shooty callus formation with a different number of shoots and hairy roots. The number of shoots was high
	Middle	Shooty friable callus formation
	Bottom	Hardy thick and off white with yellowish granular compact callus formation
15 mM	Top	Direct shoot regeneration with a distinct number of shoots
	Middle	Shooty callus formation
	Bottom	Greenish-yellow friable callus formation
25 mM	Top	Direct shoot regeneration.
	Middle	Shooty callus induction
	Bottom	Greenish yellow, friable callus formation
35 mM	Top	Direct shoot regeneration
	Middle	Shooty callus formation
	Bottom	Greenish with brown patches nodular callus formation
45 mM	Top	Friable callus formation
	Middle	Friable callus formation
	Bottom	Greenish with brown patches, friable callus formation

of callus growth with necrosis symptoms in NaCl incorporated media of cultured sugarcane. Change in callus colour from brown to black at higher salt concentrations may cause *in vitro* proliferated cells to death. This suggested that the KC-1 cultivar could tolerate NaCl concentration up to 35 mM by culturing the hypocotyls as an explant source until four weeks of culture.

The fresh weight of the cultured hypocotyl explants significantly differed ($p<0.05$) between salt-stressed and control media (Table 1). When increasing salinity concentration from 0 (control) to 45 mM, the fresh weight of hypocotyl explants decreased, and the highest reduction was observed in high salinity level (45 mM). Also, results revealed that salinity causes a significant effect on the fresh weight of the explants. In this experiment, salinity has significantly affected the *in vitro* regeneration of the tomato (KC-1). Non-salinized callus had the greater fresh weight except with 15 mM cultured explants. Callus growth was stimulated in the tomato cell lines for the lower levels of salt treatment and only reduced at the highest level of salinity stress. This is the osmotic effect of salinity. Similar results in the reduction of fresh callus weight were found at the high level of salinity stress, which agrees with FAO (2017) and disagreement with Rao and Agarwal (2000), who observed a positive response callus growth in high saline media. These results agree with a study by El-Meleigy et al. (2004), who observed a decrease in callus weight with increasing NaCl. Further, growth parameters such as fresh callus weight and shoot induction may be reduced due to less water content in the culture media with increased NaCl levels (Chamandoosti, 2007).

The colour of the callus was regularly observed during the experiment (Table 2). Callus colour in the control treatment (0 mM) ranged greenish-yellow to green (Figure 1a), while the callus colour at 45 mM ranged from greenish-yellow to brown after four weeks of culture. Green colour areas with brown colour patches were observed on 45 mM media after four weeks of inoculation. Until the four weeks, the cultured explants showed a maximum survival rate. The colour of the callus was considerably changed by increasing salt level after four weeks of culture. Brownish colour represents cell necrosis and can be used to indicate osmotic stress in tissue culture (Bouiamrine and Diouri, 2012). Reduced callus production and colour change of callus by increased NaCl concentrations agree with Sajid and Aftab (2014).

CONCLUSION

In this experiment, shoot regeneration was observed when culturing the top portion of the hypocotyl explants directly in the culture media containing a low level of NaCl. Through increasing the NaCl concentration or salt stress in the media, callus formation and fresh weight of callus were decreased. The fresh weight of callus was higher in both control (0 mM) and 15 mM salt media. Salt tolerant calli were more compact and greenish without necrotic patches. Until four weeks, the hypocotyl callus can be incubated in the salt-containing MS media, and then it can be transferred to the salt-free MS media for shoot regeneration.

REFERENCES

Aazami, M.A., Torabi, M. and Shekari, F. (2010) Response of some tomato cultivars to sodium chloride stress under in vitro culture condition. *African Journal of Agricultural Research*, 5(18): 2589-2592.

Abu-El-Heba, G.A., Hussein, G.M. and Abdalla, N.A. (2008) A rapid and efficient tomato regeneration and transformation system. *Landbauforschung Volkenrode*, 58(1/2): 103.

Agarwal, S. and Rao, A.V. (2000) Tomato lycopene and its role in human health and chronic diseases. *Canadian Medical Association Journal*, 163(6): 739-744.

Al-Khayri, J.M. (2002) Growth, proline accumulation and ion content in sodium chloride-stressed callus of date palm. *In Vitro Cellular & Developmental Biology-Plant*, 38(1): 79-82.

Amini, F. and Ehsanpour, A.A. (2006) Response of tomato (Lycopersicon esculentum Mill.) cultivars to MS, water agar and salt stress in in vitro culture. *Asian Journal of Plant Sciences*, 9(1): 170-175.

Ashraf, M. and Ahmad, S. (2000) Influence of sodium chloride on ion accumulation, yield components and fibre characteristics in salt-tolerant and salt-sensitive lines of cotton (Gossypium hirsutum L.). *Field Crops Research*, 66(2): 115-127.

Badawy, O.M., Nasr, M.I. and Alhendawi, R.A. (2008) Response of sugarcane (Saccharum species hybrid) genotypes to embryogenic callus induction and in vitro salt stress. *Sugar Tech*, 10(3): 243-247.

Bouiamrine, E.H. and Diouri, M. (2012) Response of durum wheat (Triticum durum Desf.) callus culture to osmosis-induced drought stress caused by polyethylene glycol (PEG). *Annals of Biological Research*, 3(9): 4555-4563.

Bray, E.A., Bailey-Serres, J. and Weretilnyk, E. (2000) Responses to abiotic stresses. *In: Gruissem W, Buchannan B, Jones R (Eds.), Biochemistry and molecular biology of plants*, American Society of Plant Physiologists, Rockville, MD, 1158-1249.

Chamandoosti, F. (2007) Effect of sodium chloride on establishment of callus and organogenesis in Brassica napus L. *Pakistan journal of biological sciences*, 10(21): 3880-3884.

Chaudhry, Z., Abbas, S., Yasmin, A., Rashid, H., Ahmed, H.A.B.I.B. and Anjum, M.A. (2010) Tissue culture studies in tomato (Lycopersicon esculentum) var. Moneymaker. *Pakistan Journal of Botany*, 42(1): 155-163.

Chaudhry, Z., Habib, D., Rshid, H. and Qurashi, A, S. (2004) Regeneration from various explants of in vitro seedling of tomato (Lycopersicon esculentum L., cv. Roma). *Pakistan Journal of Biological Science*, 7: 269-272.

Ehsanpour, A.A. and Fatahian, N. (2003) Effects of salt and proline on Medicago sativa callus. *Plant cell, tissue and organ culture*, 73(1): 53-56.

El-Meleigy, E.A., Gabr, M.F., Mohamed, F.H. and Ismail, M.A. (2004) Responses to NaCl salinity of tomato cultivated and breeding lines differing in salt tolerance in callus cultures. *International Journal of Agriculture and Biology*, 6(1): 19-26.

FAO, (2017) *Statistical Database*, FAO-STAT Agriculture Data. [Online]. Available:http://http://faostat.fao.org/site/567, February 26, 2017.

Gupta, P., Sharma, S. and Saxena, S. (2014). Effect of salts (NaCl and Na_2CO_3) on callus and suspension culture of Stevia rebaudiana for steviol glycoside production. *Applied biochemistry and biotechnology*, 172(6): 2894-2906.

Lokhande, V.H., Nikam, T.D. and Penna, S. (2010) Biochemical, physiological and growth changes in response to salinity in callus cultures of Sesuvium portulacastrum L. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 102(1): 17-25.

Lutts, S., Almansouri, M. and Kinet, J.M. (2004) Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. *Plant Science*, 167(1): 9-18.

Mercado, J.A., Sancho-Carrascosa, M.A., Jiménez-Bermúdez, S., Perán-Quesada, R., Pliego-Alfaro, F. and Quesada, M.A. (2000) Assessment of in vitro growth of apical stem sections and adventitious organogenesis to evaluate salinity tolerance in cultivated tomato. *Plant cell, tissue and organ culture*, 62(2): 101-106.

Osman, M.G., Elhadi, E.A. and Khalafalla, M.M. (2010). Callus formation and organogenesis of tomato (Lycopersicon esculentum Mill, CV Omdurman) induced by thidiazuron. *African Journal of Biotechnology*, 9(28): 4407-4413.

Parida, A.K. and Das, A.B. (2005) Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and environmental safety*, 60(3): 324-349.

Rashid, R. and Bal, S.S. (2010) Effect of hormones on direct shoot regeneration in hypocotyl explants of tomato. *Notulae Scientia Biologicae*, 2(1): 70-73.

Sajid, Z.A. and Aftab, F. (2014) Plant regeneration from in vitro-selected salt tolerant callus cultures of Solanum tuberosum L. *Pakistan Journal of Botany*, 46(4): 1507-1514.

Shiyamala, B. (2017) *In vitro regeneration of tomato (Lycopersicon esculentum mill.) as influenced by BAP and alt (NaCl)*. BSc thesis, Faculty of Agriculture, Eastern University, Sri Lanka.

Tewary, P.K., Sharma, A., Raghunath, M.K. and Sarkar, A. (2000) In vitro response of promising mulberry (Morus sp.) genotypes for tolerance to salt and osmotic stresses. *Plant Growth Regulation*, 30(1): 17-21.

Zaki, H.E. and Yokoi, S. (2016). A comparative in vitro study of salt tolerance in cultivated tomato and related wild species. *Plant Biotechnology*, 16-1006.

Zhang, F., Yang, Y.L., He, W.L., Zhao, X. and Zhang, L.X. (2004). Effects of salinity on growth and compatible solutes of callus induced from Populus euphratica. *In Vitro Cellular & Developmental Biology-Plant*, 40(5): 491-494.