African Journal of Biotechnology Vol. 10(62), pp. 13444-13452, 12 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1544
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Co adaptation of LiCl tolerant *Solanum tuberosum* L. callus cultures to NaCl stress

Azhar H. Shah[1], Safdar H. Shah*[1], Habib Ahmad[2], Ayesha Baig[1], Zahoor A. Swati[1], Umm-e-Aiman[1], Israr ud Din[1], Qudsia Khalid[1] and Abrar Hussain Shah[3]

[1]Institute of Biotechnology and Genetic Engineering,
[2]Botany Dept Hazara University, Pakistan.
[3]Horticulture Dept KPK Agricultural University, Peshawar.

Accepted 11 August, 2011

In this research, co-adaptation of the Calli of *Solanum tuberosum*, raised from petioles, to the presence of lithium (LiCl) and sodium chloride (NaCl) was studied. The cultures were adapted with LiCl in the absence of an osmotic stress and the response of adapted and unadapted calli to salinity was investigated. Undifferentiated callus growth was induced in *S. tuberosum* by the addition of 2 mg/l 2,4 dichlorophenoxy acetic acid (2,4-D), 0.25 mg/l kinetin to Murashige and Skoog medium. Subcultures were subjected to an incremental increase in LiCl to obtain adapted lines. Adapted and undapted calli were grown with LiCl and NaCl and the tissue content of Na^+, K^+, Ca^{2+}, Mg^{2+} and proline levels were determined. Either 40 mM LiCl or 100 mM NaCl inhibited unadapted calli by more than 50%, while adapted calli grew normally under these conditions. The adapted calli exhibited a lower K^+ content with or without salt and showed a lower accumulation of Na^+ at 100 mM NaCl. The tissue K^+ and Mg^{2+} contents decreased and their proline levels increased with salinity. A co-adaptation phenomenon is induced by LiCl that involves a regulation of K^+ and Na^+ contents and an accumulation of proline, which also brings about tolerance to osmotic effects of salt. This data is highly useful for devising breeding and molecular modification strategies for stress tolerance.

Key words: Cations, proline, osmotic adjustment, salt tolerance, *Solanum tuberosum*.

INTRODUCTION

Soil salinity is a major abiotic factor affecting about 40% of irrigated land and 20% of arable land globally (Rhodes and Loveday, 1990). Improving salt tolerance in crop plants remains a difficult task. Due to our understanding of salt tolerance mechanisms in plants, salt tolerance remains limited and the nature of salt stress is complex causing osmotic and ionic disruptions as well as affecting plant nutrition and developmental processes. These edaphic factors interact closely with growth phases of plants and are also influenced by seasonal fluctuations (Orcutt and Nilsen, 2000; Pardo et al., 2007). Naturally, the plant responses to these multifold effects are also complex and diverse (Yeo, 1998; Orcutt and Nilsen, 2000).

Currently, the main focus of attention in plant response to salt stress has been on the elucidation of metabolic pathways associated with salt tolerance mechanisms at the cellular and whole plant level (Greenway and Munns, 1980; Hasegawa et al., 2000; Tester and Davepott, 2003). One of the important mechanisms of salinity tolerance in plant cells is the ionic homeostasis maintenance of cellular sodium at a low level because sodium toxicity is the key factor affecting plant growth under saline conditions (Greenway and Munns, 1980). Many salt tolerant plants are able to avoid intracellular Na^+ excess by selective permeability of plasma membrane and tonoplast, where a low level of cytosolic Na^+ is ensured under high salt concentrations (Nakayama et al.,

*Corresponding author. E-mail: drsfadarshah@yahoo.co.in. Tel: 092 91 9916580, 092 3225164286.

Abbreviations: 2,4-D, 2,4-Dichlorophenoxy acetic acid; **RGR,** relative growth rate; **LiCl,** lithium chloride; **NaCl,** sodium chloride.

2005). The body of data on salt balance in plants under saline conditions appears consistent with the concept of Na^+ exclusion from metabolic sites and thus offers a useful means for devising strategies of raising salt tolerant agricultural crops. Furthermore, using isogenic lines of crop plants provides a reliable source for monitoring metabolic alterations under saline conditions in a defined plant system. Moreover, the tissue culture techniques readily available for many crop plants offer a means of studying plant responses to salinity without the complications of growth stage specific variations (Shah et al., 1993; Hasegawa et al., 1994). Also, the regeneration of adapted cell lines has the potential to provide a source of plants with improved tolerance (Wincove, 1991, 1996; Chaudhary et al., 1994).

Lithium, an analogue of sodium, has been used elsewhere for studying ionic toxicity under saline conditions (Shah et al., 2002; Nakayama et al., 2005). Lithium is highly toxic to plants and in its presence, the ionic toxicity is by far the most predominant factor affecting the plant growth (Shah et al., 2002; Tester and Davenport, 2003; Nakayama et al., 2005). It can therefore be suggested that selecting plant cell lines in the presence of LiCl provides a useful mean of inducing ionic adaptation in plants under salt stress.

Solanum tuberosum can be readily raised by *in vitro* technology that has been employed extensively for studying its stress responses at different developmental stages (Martinez et al., 1996; Rahnama and Ebrahimzadeh, 2004). This study was aimed at raising *S. tuberosum* callus cultures adapted to resist the presence of lithium chloride (LiCl) in order to investigate the responses of LiCl adapted tissue cultures to high sodium chloride (NaCl) concentrations. The study also looked at the cationic balance and proline accumulation in calli grown at different ionic concentrations.

MATERIALS AND METHODS

Establishment of callus cultures

Petiole explants of *S. tuberosum* cv cardinal were surface sterilized with 50% bleach for 5 min followed by five washes with sterile distilled water. Calli were induced by inoculation of explants onto 50 ml semisolid (Murashige and Skoog, 1962) medium in 200 ml Erlenmeyer flasks. The medium was supplemented with 2 mg/l 2, 4-D, 0.25 mg/l kinetin, 30 g/l sucrose; pH was adjusted to 5.8 and solidified with 9 g/l agar. For calli induction and maintenance cultures were incubated in the dark at 28 ± 1°C for 28 days. Following the 5th passage, rapidly growing friable calli were subjected to an incremental increase in LiCl concentration in order to select resistant cell lines. Concurrently, control lines were maintained in the absence of LiCl. All cultures were prepared and incubated using aseptic procedures.

Selection of LiCl tolerant lines

In our preliminary studies, *S. tuberosum* callus cultures were able to grow, albeit at a reduced rate, with up to 75 mM LiCl. At 40 mM LiCl, cultures were able to grow at a rate of 40 to 50% of normal cultures and therefore 40 mM LiCl was selected as a suitable concentration for adapting cultures to LiCl (Shah, 2006). A multi-step procedure was used to raise adapted lines (Shah et al., 2002). The sequence of increasing LiCl concentration was; 10 (1 passage), 20 (2 passages), 30 (2 passages) and 40 mM LiCl (5 passages). The stability of tolerance to LiCl was tested by subculturing adapted calli without LiCl for two successive passages and growing them back with 40mM LiCl for 28 days. As with 40 mM LiCl, a 40 to 50% growth reduction also occurred in the presence of 100 mM NaCl; these two salt concentrations were therefore selected for a comparison of *S. tuberosum* responses to Li^+ and Na^+ salts. .

Harvesting

The tissue mass from each flask was collected as a single callus culture. Care was taken to remove the solid media particles including the callus cells adhering to the medium from the base of callus tissue. The callus growth was estimated on the fresh weight basis and the fresh sample was fractionated into two parts for proline estimation and atomic absorption spectrometric analysis.

Relative growth rates

The relative growth rate (RGR) of calli was estimated on the fresh weight (FW) basis (Shah et al., 1990) using the following formula:

$$RGR\ (week)^{-1} = (Ln\ (FW_{Final}) - Ln\ (FW_{Initial})\ /Weeks$$

The index of tolerance was calculated as:

$$\text{Index of Tolerance} = RGR_{treatment} / \text{Mean RGR}_{\text{respective media}}$$

Respective media refer to control medium for growth of unadapted calli and medium containing 40 mM LiCl for adapted calli.

Measurement of Na^+, K^+ and Mg^{2+} contents

Oven dried calli samples digested in nitric acid were serially diluted in 10% HNO_3 (Hodson et al., 1981). The concentrations of Na^+, K^+ and Mg^{2+} were determined by atomic absorption spectrometry using a Perkin Elmer A Analyst 700 system.

Determination of free proline

Free proline content in fresh tissue was quantified by the method of Bates et al. (1973); 0.1 g of tissue was homogenized in 3% sulfosalicylic acid and the homogenate filtered through Whatman # 2 filter paper was assayed for proline. L-Proline obtained from Sigma was used as a standard.

RESULTS

In vitro selection of LiCl adapted calli

In S. tuberosum, the hormonal supplementation of MS media needed for the development of undifferentiated calli was 2 mg/l 2,4-D and 0.25 mg/l kinetin. Under these conditions, calli initiation occurred in about 9 to 10 days and fast growing pale yellow calli were isolated for further studies on day 28. Subject to an incremental increase in

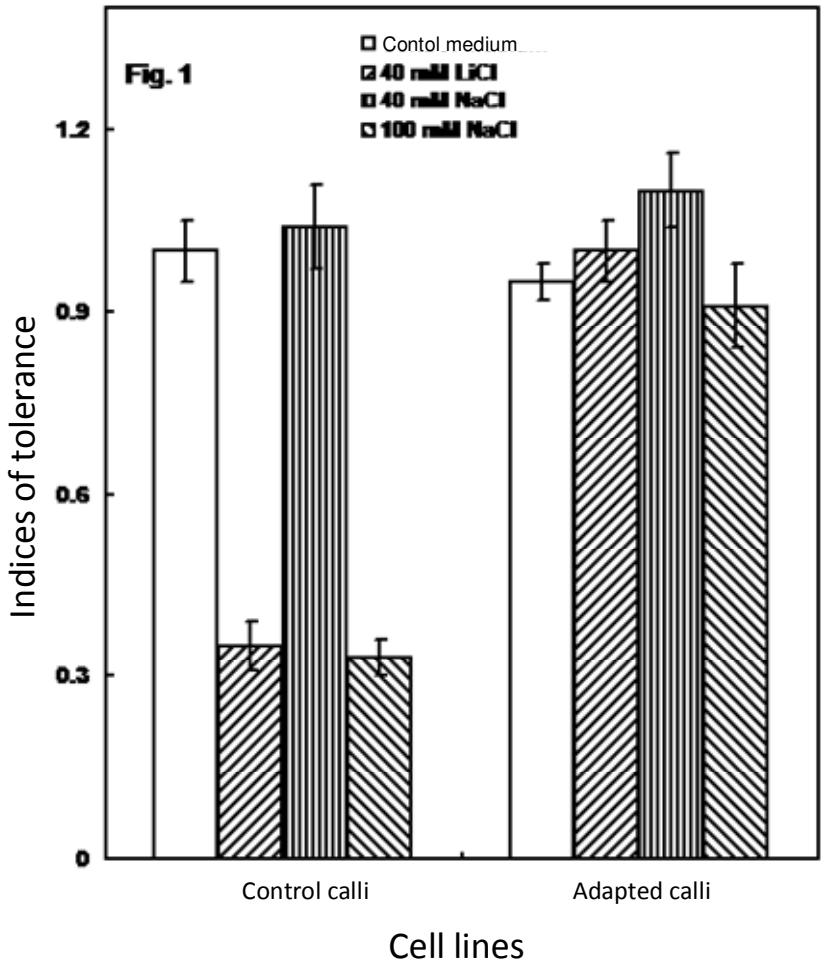

Figure 1. Indices of tolerance to NaCl and LiCl stresses. LiCl adapted and unadapted calli *S. tuberosum* cv. cardinal were incubated for four weeks on MS semi-solid media of varying ionic concentrations. □, Control medium; ▨, medium containing 40 mM LiCl; ▥, medium containing 40 mM NaCl; ▧,medium containing 100 mM NaCl. Vertical bars represent the mean values of 5 replicates ± standard error.

the LiCl concentration to 40 mM, calli growth appeared with a friable appearance that was sometime fractionated into two to three independently growing pieces. The lines selected in the presence of 40 mM LiCl were tested for two successive passages for the stability of their tolerance to lithium and those which were growing normally with 40 mM LiCl were selected as LiCl adapted lines.

Growth rates and indices of tolerance

The relative growth rate (RGR) of unadapted callus culture growing on MS control medium was 0.72±0.04 per week, whereas the RGR of LiCl adapted calli growing in the presence of 40 mM LiCl was 0.74±0.06 per week. These RGR values of control and LiCl adapted calli were used as reference points for calculating indices of tolerance of adapted and unadapted calli at different ionic concentrations (Figure 1). In *S. tuberosum*, unadapted calli showed a greater than 60% inhibition in growth upon transfer to media with 40 mM LiCl, while no inhibition occurred in these calli with a similar ionic increase of 40 mM NaCl (Figure 1). However these unadapted calli suffered a growth inhibition of about 70% when the NaCl concentration of the medium was raised to 100 mM. On the other hand, adapted line showed enhanced tolerance at 40 mM NaCl with almost no decline in tolerance at LiCl and at 100 mM NaCl stresses, respectively.

Measurement of Na^+, K^+, and Mg^{2+} contents

Without the addition of NaCl to MS media, the calli Na^+

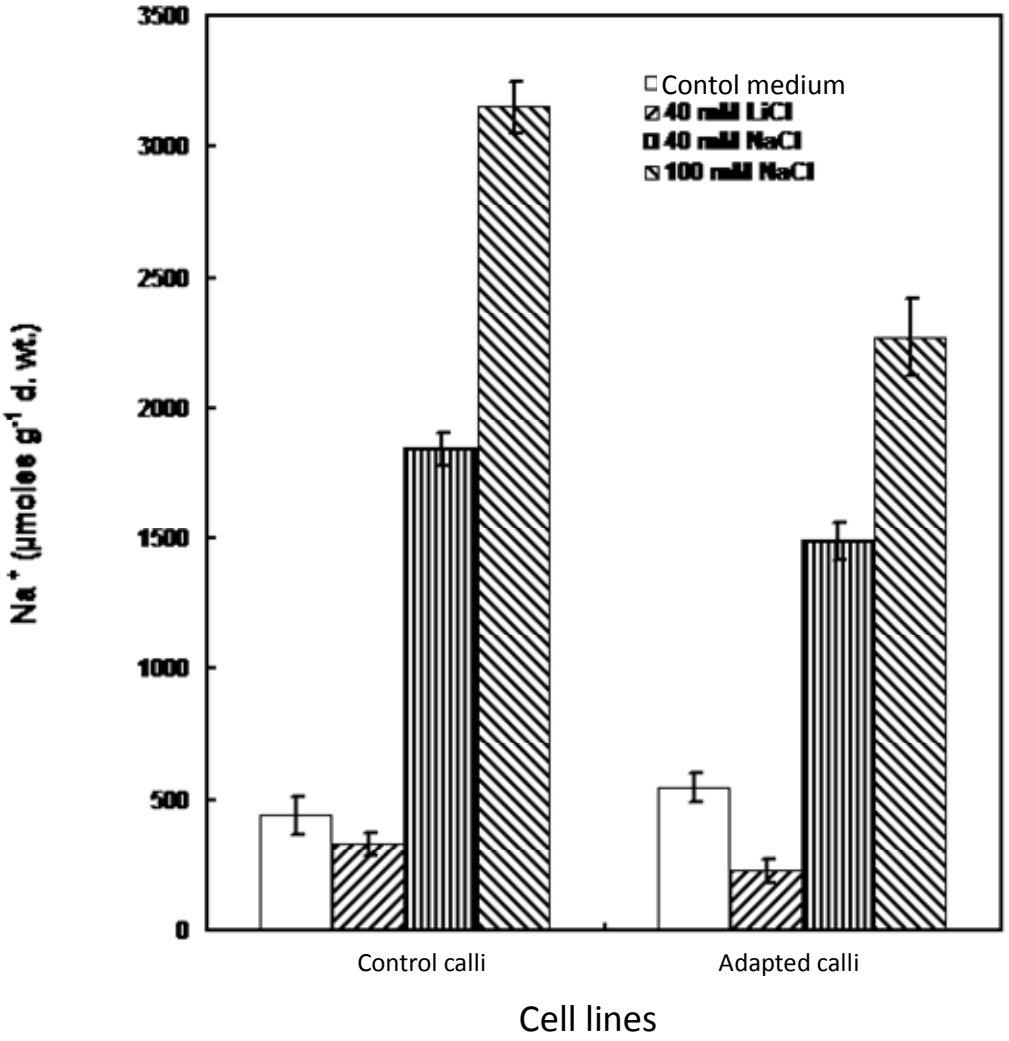

Figure 2. Effect of NaCl and LiCl concentration on the Na^+ content of LiCl adapted and unadapted calli of *S. tuberosum* cv. Cardinal. Calli grown for four weeks on MS semi-solid media were harvested and analyzed for Na^+ content by atomic absorption spectrometry. □, Control medium; ▨, medium containing 40 mM LiCl; ▥, medium containing 40 mM NaCl; ▧,medium containing 100 mM NaCl. Vertical bars represent the mean values of five replicates ± standard error.

content was low in the control media and declined even further when LiCl was added to the media. An addition of NaCl (40 and 100 mM NaCl) in the medium resulted in a significant increase in Na^+ contents of both the lines but the increase was significantly greater in unadapted than adapted calli (Figure 2).

The K^+ content of calli grown on MS control media without ionic alterations was about 30% lower in the adapted than in the unadapted calli. The K^+ content of all callus cultures decreased when either LiCl or NaCl was added to the media, with declines in K^+ similar at 40 mM LiCl and 100 mM NaCl (Figure 3). A Na^+/K^+ ratio of about 0.2 was observed in the control and 40 mM LiCl media, however, this ratio increased markedly with the addition of NaCl to the media and at 100 mM NaCl the ratio was about 1.47±0.1 and 1.68±0.7 in adapted and unadapted calli, respectively (Figure 4).

The presence of LiCl in the media brought about a drastic decrease in the Mg^{2+} content of both adapted and unadapted calli (Figure 5). The presence of 100 mM NaCl also brought about a significant decrease in the callus Mg^{2+} content, where the decline in adapted calli was less pronounced than that in the unadapted calli (Figure 5).

Proline accumulation

The proline content of unadapted calli increased with the addition of either LiCl or NaCl to the media; the levels of proline accumulation at 40 mM LiCl and 100 mM NaCl

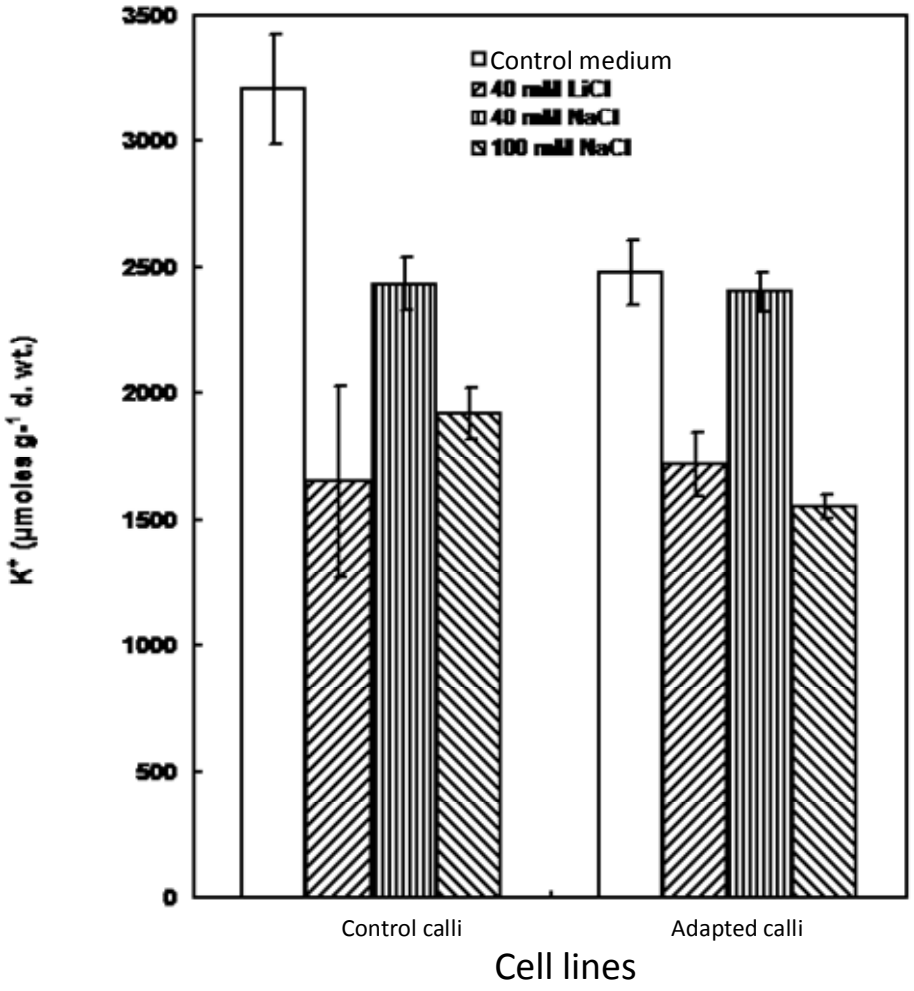

Figure 3. Effect of NaCl and LiCl concentration on the K^+ content of LiCl adapted and unadapted calli of *S. tuberosum* cv. Cardinal. Calli grown for 4 weeks on MS semi-solid media were harvested analyzed for K^+ content by atomic absorption spectrometry. □, Control medium; ▨, medium containing 40 mM LiCl; ▥, medium containing 40 mM NaCl; ▧, medium containing 100 mM NaCl. Vertical bars represent the mean values of five replicates ± standard error.

were significantly higher (Figure 6). In contrast, adaptation to LiCl resulted in a decline in proline contents, however, when adapted line was grown on the control and NaCl supplemented medium, proline level significantly increased (Figure 6).

DISCUSSION

This study demonstrates the existence of co-adaptation mechanisms in a crop plant where an exposure to sublethal levels of LiCl led to an enhancement of the plant's ability to cope with NaCl excess. Lithium has been employed for the induction of salt tolerance in other crop species (Shah et al., 2002). Lithium is known to inhibit growth at concentrations as low as 20 mM (Hodson et al., 1981), its impact on plant growth therefore appears ionic rather than osmotic in the nature. In *S. tuberosum*, undifferentiated callus cultures were able to withstand a shock of 75 mM LiCl and its growth rate after a 40 mM LiCl shock was about 50% of normal cultures. However, the calli were able to adapt without any growth inhibition at 40 mM LiCl, when the concentration of LiCl was increased gradually in order to allow time for calli to make an adjustment to ionic increases. The difference between adapted and unadapted calli was apparently associated with the ionic effects of 40 mM LiCl, as an isosmotic concentration of 40 mM NaCl had no effect on the growth of *S. tuberosum* calli. It is interesting to note here that an adaptation to 40 mM LiCl in *S. tuberosum* calli led to a tolerance in calli to a much higher level of NaCl concentration, as adapted calli suffered no growth

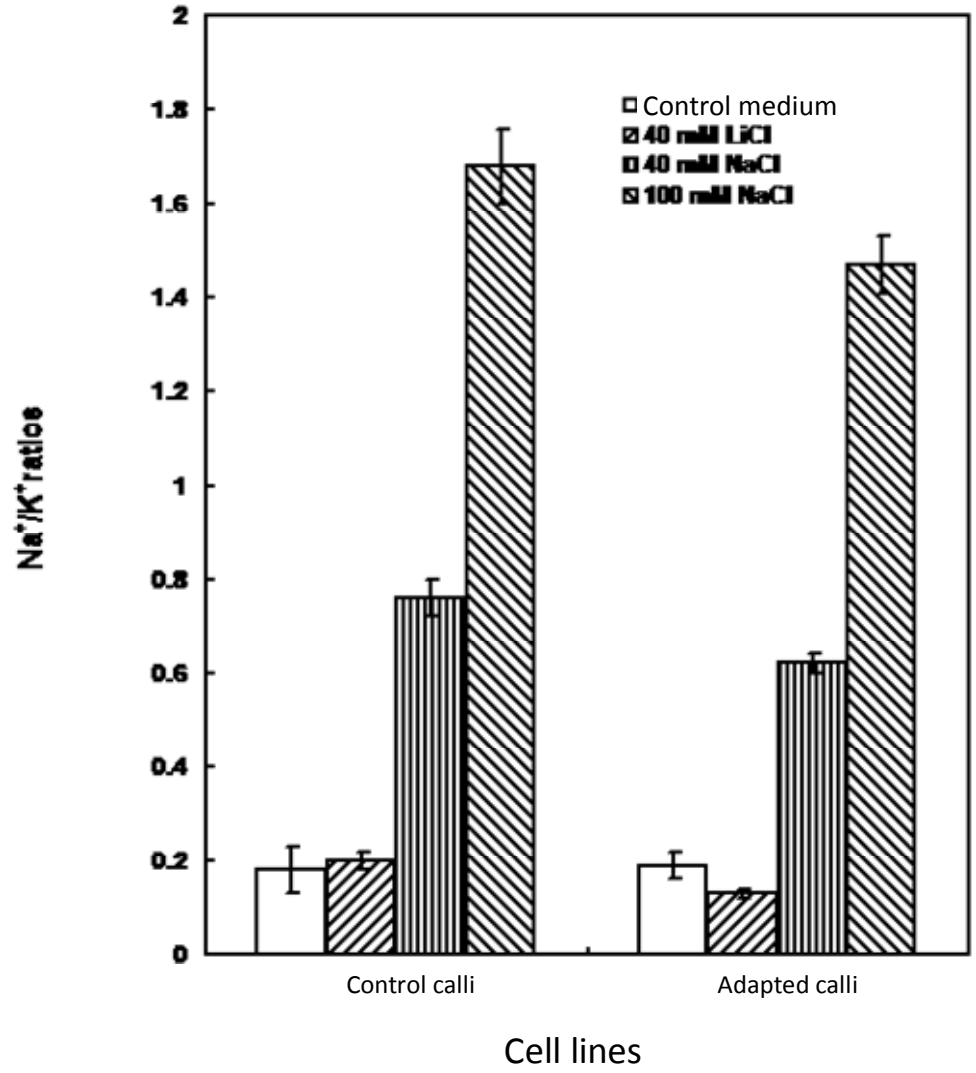

Figure 4. Effect of NaCl and LiCl concentration on the Na^+/K^+ ratio of LiCl adapted and unadapted calli of *S. tuberosum* cv. Cardinal. The values presented on Na^+/K^+ are based on the data shown in Figures 3 and 4. □, Control medium; ▨, medium containing 40 mM LiCl; ▥, medium containing 40 mM NaCl; ▧,medium containing 100 mM NaCl. Vertical bars represent the mean values of five replicates ± standard error.

inhibition at 100 mM NaCl. In plants, adaptation to salt stress, a sequential pattern of response was proposed by Munns (2005), where the first phase involves an osmotic adjustment and second phase deals with salt specific effects. Our data suggest that an adaptation to ionic toxicity can also occur in the absence of an osmotic stress. In *S. tuberosum*, the adaptation to ionic toxicity appears to be strongly associated with the regulation of ion uptake, where the regulatory processes appear to involve share mechanism for the avoidance of a salt excess in the cell, as LiCl adapted calli were also able to tolerate high NaCl concentrations. It is pertinent to note here that the induction of regulatory mechanisms under ionic stress also enabled the calli to cope with the osmotic component of salt stress as calli adapted to 40 mM LiCl were able to adjust and grow normally at 100 mM NaCl, with an osmotic effect 2.5 times higher than 40 mM LiCl.

In *S. tuberosum*, the salt balance under saline conditions appears to involve an alteration in the ionic composition with changes in the levels of Na^+ and K^+ as the key response. The regulation of sodium accumulation appears to be a major mechanism of salt tolerance in *S. tuberosum*. However, the induction of regulatory pathway for salt balance does not appear to be specifically dependant upon the presence of Na^+, as an adaptation to LiCl also enabled the calli to restrict the accumulation of Na^+ under saline conditions. A common regulatory mechanism for Na^+ and Li^+ appears to operate in *S. tuberosum*, which in part may explain the occurrence of

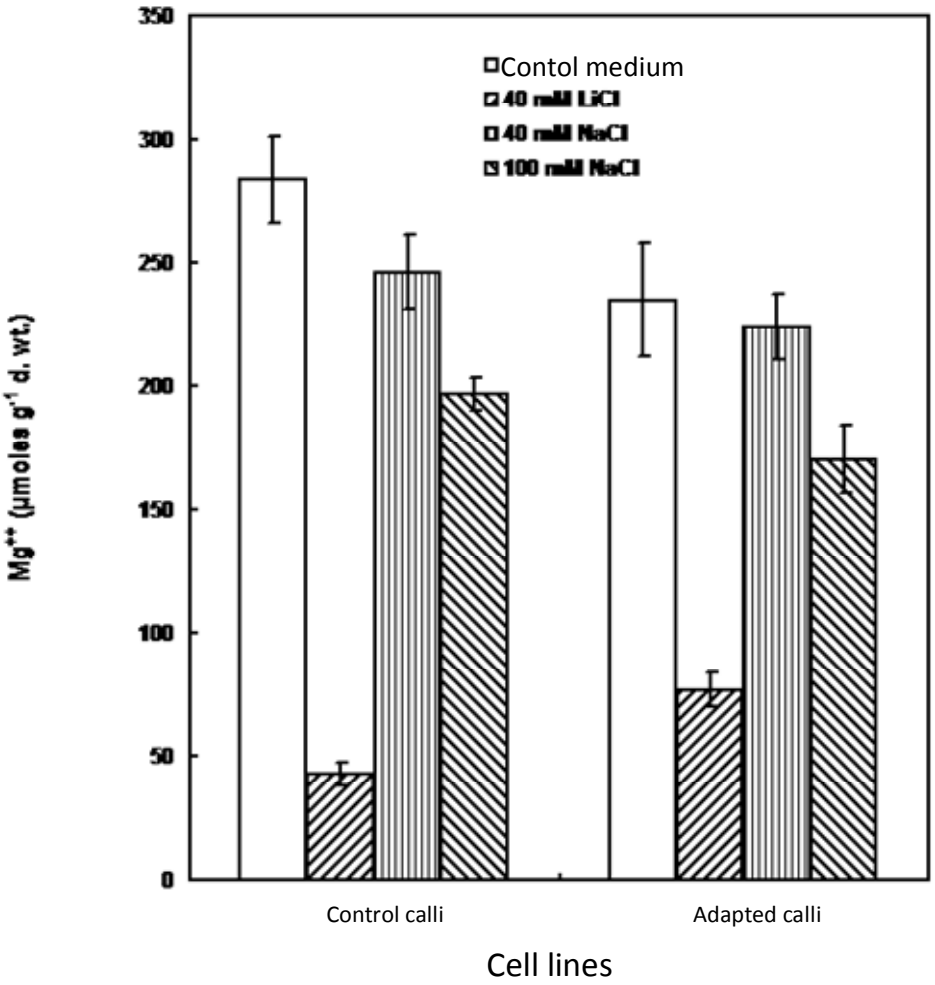

Figure 5. Effect of NaCl and LiCl concentration on the Mg^{2+} content of LiCl adapted and unadapted calli of *S. tuberosum* cv. Cardinal. Calli grown for four weeks on MS semi-solid media were harvested analyzed for Mg^{2+} content by atomic absorption spectrometry. □, Control medium; ▨, medium containing 40 mM LiCl; ▥, medium containing 40 mM NaCl; ▧,medium containing 100 mM NaCl. Vertical bars represent the mean values of five replicates ± standard error.

co-adaptation to LiCl and NaCl in this crop plant. Saline conditions are generally known to cause a decline in the tissue K^+ content (Maathuis and Amtmann, 1999) and in *S. tuberosum*, the potassium content of calli were lowered by the presence of LiCl and NaCl, indicating an ionic replacement of K^+ by Na^+ and Li^+ ions under these conditions. In glycophytes, the tissue ionic balance in terms of Na^+/K^+ ratio is regarded as an indicator of salt tolerance, where the tolerant plants are shown to maintain a lower Na^+/K^+ ratio under saline conditions (Shah et al., 1993; Chaudhary et al., 1997; Chinnusamy et al., 2005). In the glycophytic *S. tuberosum*, the Na^+/K^+ ratio at 100 mM NaCl was clearly lower in the adapted calli than the unadapted ones. However, in *S. tuberosum*, an adaptation to excess salt cannot be simply explained in terms of a maximum retention of K^+ under saline condition. The LiCl adapted calli after undergoing a passage in the absence of salt exhibited tissue K^+ content about 30% lower than that in unadapted calli. It appears that the adapted calli were adjusted to maintain normal turgor and growth with a lower level of K^+ accumulation. In *S. tuberosum,* the Mg^{2+} content of calli also showed a significant decline in the presence of ionic excess in the medium. The effect of Li^+ on Mg^{2+} was particularly marked reducing the Mg^{2+} content by more than 80% in unadapted calli and by about 60% in adapted calli. As with its effect on K^+ uptake (Demidchik and Maathuis, 2007), Li^+ appears to be an effective blocker of Mg^{2+} uptake.

In plants under saline conditions, toxic ions are generally considered to be sequestered in the vacuolar compartment, and non ionic molecules such as imino

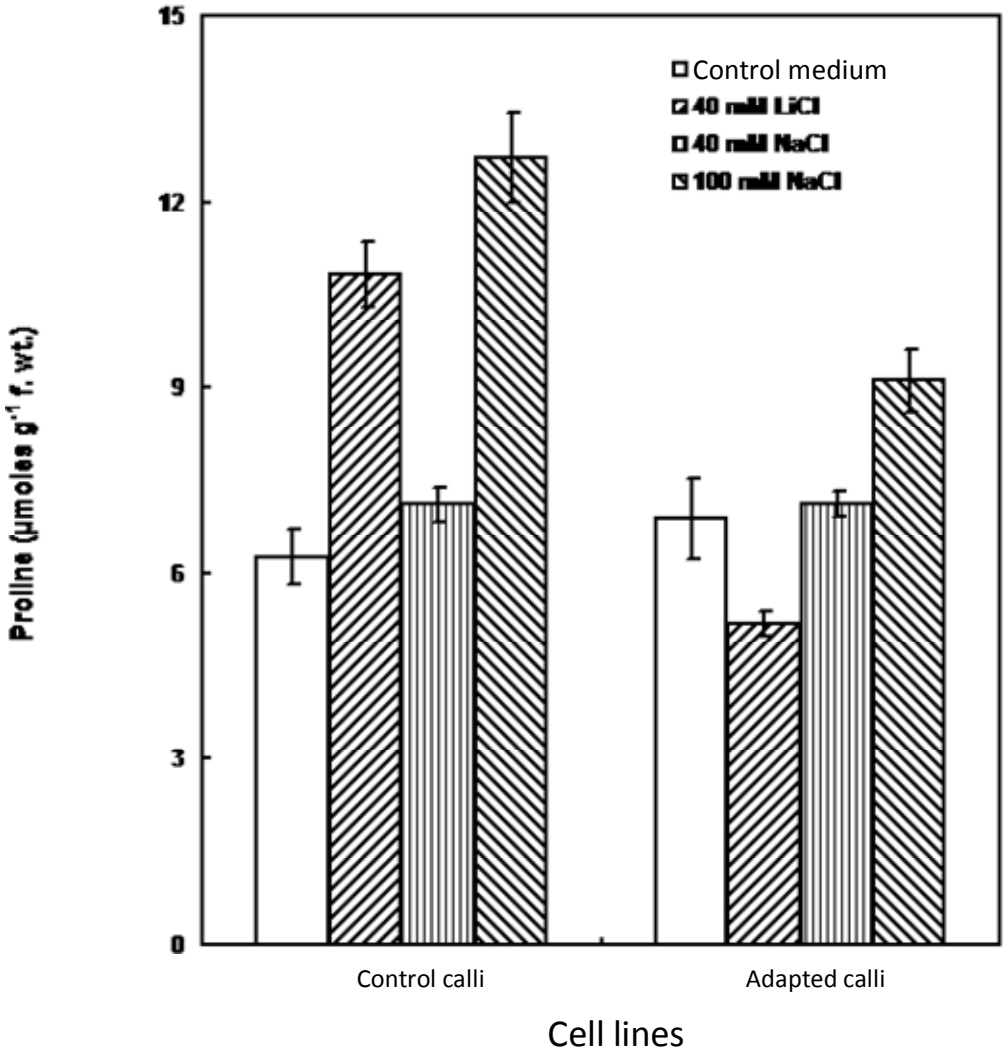

Figure 6. Effect of NaCl and LiCl concentration on proline accumulation in unadapted and LiCl adapted calli of *S. tuberosum* cv. Cardinal. Calli grown for 4 weeks on MS semi-solid media were extracted and analyzed for free proline. □, Control medium; ▨, medium containing 40 mM LiCl; ▥, medium containing 40 mM NaCl; ▧,medium containing 100 mM NaCl. Vertical bars represent the mean values of five replicates ± standard error.

acid proline, quaternary ammonium compounds and sugar alcohols are accumulated in the cytoplasm to make the necessary osmotic adjustment (Mahajan and Tuteja, 2005). In *S. tuberosum*, proline has been identified as an important osmoticum under saline conditions (Martinez et al., 1996; Rahnama and Ebrahimzadeh, 2004). Our data show a significant increase in the calli proline content in the presence of both LiCl and NaCl, where the levels of proline were higher in unadapted than adapted calli. These values of proline accumulation in adapted and unadapted calli appear consistent with the total salt accumulation in these tissues. It appears that in comparison to unadapted calli, the adapted ones by maintaining a lower ionic load will need a lower level of proline to make the intracellular osmotic adjustment between the cell vacuole and the cell cytoplasm.

A question can be raised here as to why the unadapted calli with a higher accumulation of sodium and proline and thus apparently with a lower tissue water potential are not able to tolerate salt stress better than their LiCl adapted counterparts. It can be argued that in an internally compartmentalized plant tissue, it is not simply the total salt load that determines its water relations with respect to the external environment. A favorable osmotic adjustment in a plant requires a fine balance between various cell compartments for maintaining a water potential gradient for the cell turgor and growth under saline conditions. In *S. tuberosum*, maintaining the salt load to a manageable level is an important prerequisite of cellular osmotic adjustment and tolerance to salinity.

This study proves the usefulness of employing *in vitro* tissue culture systems for studying responses of plants to important physiological factors such as abiotic stresses and environmental toxicity. In this study, the exposure of undifferentiated callus culture to LiCl led to an early induction of mechanisms associated with tolerance to this highly toxic cation and also revealed the presence of co-adaptation mechanisms allowing the calli adapted to 40 mM LiCl to tolerate much high levels of NaCl. The cross adaptation in plants to environmental stresses is becoming an important tool for enhancing tolerance in crop plants and the data presented in this study should provide a useful insight for devising breeding and molecular modification strategies for stress tolerance.

ACKNOWLEDGEMENTS

The work carried out in this study was a part of a M. Phil Program of the first author and was supported financially by the Higher Education Commission (HEC) grants. The technical assistance of Mr. Sajjad Ahmad is gratefully acknowledged. Cardinal cultivar used in the study was a gift from Potato Research Centre Abbottabad

REFERENCES

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. Plant Soil. 39: 205-207.

Chinnusamy V, Jagendorf A, Zhu JK (2005). Understanding and improving salt tolerance in plants. Crop Sci. 45: 437-448.

Chaudhary MT, Wainwright SJ, Merrett MJ, Alam MS (1994). Salt tolerant plants of lucern (*Medicago media* Pers) regenerated from salt selected suspension cultures. Plant Sci. 98: 97-102.

Chaudhary MT, Wainwright SJ, Merrett MJ (1997). Ion accumulation in lucern plants regenerated from salt-adapted suspension cultures compared with recultured cells from these plants. Plant. Cell Rep., 17: 145-149.

Demidchik VA, Maathuis FJ (2007). Physiological role of nonselective cation channels in plants from salt stress to signaling and development. New Phytol. 175: 387-404.

Greenway H, Munns R (1980). Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31: 149-190.

Hasegawa PM, Bresan RA, Nelson DE, Samaras Y, Rhodes D (1994). Tissue culture in the improvement of salt tolerance in soil minerals stresses: Springer Verlage New york, London.

Hasegawa PM, Bresan RA, Zhu JK, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Physiol. Plant Mol. Biol. 51: 463-499.

Hodson MJ, Smith MM, Wainwright SJ, Opik H (1981). Cation cotolerance in a salt tolerant clone of *Agrostis stolonifera* L. New Phytol. 90: 253-261.

Maathuis FJM, Amtmann A (1999). K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. Ann. Bot. 84: 123-133.

Mahajan S, Tuteja N (2005). Cold salinity and drought stress: An overview. Arch. Bioch. Bioph. 444: 139-154.

Martinez CA, Maestri M, Lani EG (1996). In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. Plant Sci. 116: 177-184.

Munns R (2005). Genes and salt tolerance: bringing them together. New Phytol. 167: 645-663.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant. 15: 437-447.

Nakayama H, Horie T, Yonamine I, Shinmyo A, Yoshida K (2005). Improving salt tolerance in plant cell. Plant Biotech. 22: 477-487.

Orcutt DM, Nilsen ET (2000). Phytotoxicity and soil pollution: Heavy metals andxenobiotics. In: John Wiley & Sons, I. (eds). The physiology of plants understress: Soil. Biotic. factors. New York, Chichester, Weinheim, Brisbane, Singapore, Toronto. pp. 481-517.

Pardo JM, Cubero B, Leidi EO, Quintero FJ (2007). Alkali cation exachangers: role in cellular homeostasis and stress tolerance. J. Expt. Bot. 57: 1181-1199.

Rahnama H, Ebrahimzadeh H (2004). The effect of NaCl on proline accumulation in potato seedlings and calli. Acta Physiol. Plant. 26: 263-270.

Rhoades JD, Loveday J (1990). Salinity in irrigated agriculture. In American Society of Civil Engineers, Irrigation of Agricultural Crops (Steward BA and Nielsen DR eds), Am. Soc. Agron. Mono. 30: 1089-1142.

Shah AH (2006). Coadaptation and ionic homeostasis in cell lines of Solanum tuberosum LM. Phil Thesis, Agric.I Uni. Peshawar, Pakistan.

Shah SH, Tobita S, Shono M (2002). Cation co tolerance phenomenon in cell cultures of *Oryza sativa* adapted to LiCl and NaCl. Plant Cell, Tissue Org. Cult. 71: 95-101.

Shah SH, Wainwright SJ, Merrett MJ (1993). Cation co tolerance in callus cultures of *Medicago sativa* L. tolerant to sodium chloride. Plant Sci. 89: 81-84.

Shah SH, Wainwright SJ, Merrett MJ (1990). The interaction of sodium and calcium chloride and light on growth potassium nutrition and proline accumulation in callus cultures of *Medicago sativa* L. New Phytol. 116: 37-45.

Tester M, Davenport R (2003). Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. 91: 503-527.

Wincove I (1996). Characterization of rice (*Oryza sativa L.*) plant regenerated from salt tolerant cell lines. Plant Sci. 113: 105-111.

Wincove I (1991). Characterization of salt tolerant alfalfa (*Medicago sativa L.*) plants regenerated from salt tolerant cell lines. Plant. Cell. Rep., 10: 561-564.

Yeo A (1998). Molecular biology of salt tolerance in the context of whole plant physiology. J. Expt. Bot. 49: 913-929.