

*Full Length Research Paper*

# The phenomenon of cross tolerance in osmotically and ionically adapted rice (*Oryza sativa* L.) cell lines

Azhar H. Shah<sup>1</sup>, Safdar H. Shah<sup>1\*</sup>, Habib Ahmad<sup>2</sup>, Zahoor A. Swati<sup>1</sup>, Abrar H. Shah<sup>1</sup>, M. Afzal<sup>2</sup>, Umm-e-Aiman<sup>1</sup> and Q. Khalid<sup>1</sup>

<sup>1</sup>Institute of Biotechnology and Genetic Engineering, KPK, Agricultural University Peshawar, Pakistan

<sup>2</sup>Hazara University, Mansehra, Pakistan

Accepted 23 December, 2011

In the present study, cell lines of local rice (*Oryza sativa* L.) cv. Swat-1 were incrementally adapted to osmotic [20% Poly ethylene glycol (PEG)] and ion specific (20 mM LiCl) stresses to investigate the phenomenon of cross tolerance. The tolerances of adapted lines were stable up to six generation on stress free medium. Both adapted cell lines were highly tolerant to either osmotic or ionic stresses while, the relative growth rates (RGR) of unadapted cells line decreased to about 95% at 20 mM LiCl and 20% polyethylene glycol, respectively. On subjection to temperature stress, the relative growth rates of unadapted cells line completely ceased, in contrast, both the adapted lines were found to be highly tolerant to low (18°C) and high temperature (36°C) stresses, respectively. At optimum temperature (27°C), proline content of unadapted and LiCl adapted lines were comparable (2  $\mu\text{moles g}^{-1}$  fresh weight), while polyethylene glycol adapted cell line had 17 time higher proline (35  $\mu\text{mole g}^{-1}$  fresh weight) content. Under temperature stresses, the proline level of both the adapted lines increased significantly but the relative rate of increase was greater in LiCl adapted line than PEG adapted line. The tolerance of adapted cell lines to opposite stresses (osmotic or ionic) and to temperature stress (low and high) tangibly revealed that the presence of common stressor in the environment is not simply the cause of induction of cross tolerance, rather it seems to be the adaptations of software that facilitates induction of this phenomenon.

**Key words:** Cross tolerance, growth, LiCl, polyethyleneglycol (PEG), proline.

## INTRODUCTION

Plants growing in the natural environments are frequently hindered from expressing their full genetic potential, because plant seldom experience optimal environmental conditions and it is a norm that they experience some sort of stresses during life cycle (Chrispeels and Sadava, 2003; Fosket, 1994). Being sessile, plants are incapable of escaping from environmental insults; therefore, for survival, plants develop resistance mechanisms against environmental

stresses mainly through the changes in physiology at the cellular level. Among the abiotic stresses, drought and salinity are the major ones and these environmental concerns affect plants more than commonly thought (Serrano et al., 1999).

In many plants, changes in the environments induce tolerance to extreme conditions and to other stresses. The tolerance of plants to adapt/acclimate to a range of different stresses after exposure to one specific stress is referred to as cross tolerance (Pastori and Foyer, 2000; Alexieva et al., 2003). This is evident from existence of considerable overlap among plant responses to salinity, drought, low and high temperature stresses, in particular (Ryu et al., 1995; Kreps et al., 2002; Wang et al., 2003; Sreenivasulu et al., 2007). The appearance of the phenomenon of cross tolerance is generally seen as

\*Corresponding author. E-mail: [drsfadarshah@yahoo.co.in](mailto:drsfadarshah@yahoo.co.in). Tel: 092 91 9916580 or 092 3225164286.

**Abbreviations:** 2,4-D, 2,4 Dichlorophenoxy acetic acid; PEG, polyethyleneglycol; RGR, relative growth rate; SCV, sedimented cell volume.

the consequence of presence of the common stressor in the environment for example salinity, drought and temperature stress leads to physiological drought stress, therefore, tolerance against any of these stresses confers tolerance to other stresses (Bowler and Fluhr, 2000; Mahajan and Tuteja, 2005; Wahid et al., 2007). There is little information on the relationship between occurrences of cross tolerance for abiotic stresses in the absence of common stressor in the environment. The present study was aimed at adapting cell lines of *Oryza sativa* L. cv. Swat-1 to osmotic (poly ethylene glycol) and ion specific (LiCl) stress to elucidate the response of adapted lines to each other and to temperature stress with the objectives: Whether presence of common stressor in the environment is imperative for induction of cross tolerance or some other underlying mechanisms also play role in induction of this mechanism(s).

## MATERIALS AND METHODS

Calli were induced from mature seed of rice (*O. sativa* L.) cv. Swat-1. Dehulled seeds were surface sterilized in 70% ethanol for 30 s followed by a 15 min washing with 70% bleach. After five washes with sterilized distilled water, seeds were incubated onto Murashige and Skoog (MS) medium (1962) supplemented with 2 mg/l 2,4 -D, 0.25 mg<sup>-1</sup> kinetin, 2 g<sup>-1</sup> casein hydrolysate, 30 g<sup>-1</sup> sucrose and pH was adjusted to 5.8 and solidified with 9 g<sup>-1</sup> agar. All the cultures were incubated in the dark at 27 ± 2°C. Following fourth subculture of 28 days each, rapidly growing friable calli were sub-cultured and used for suspension cultures. Suspension cultures were established by inoculation of calli into 50 ml liquid MS medium in 200 ml Erlenmeyer flasks. The cultures were incubated at 100 rpm in shaking incubator at 27 ± 2°C. After two weeks, dense suspensions were sub-cultured into fresh medium by decanting to about 1/10 dilution every 10 to 14 days depending on growth of suspensions.

### Selection procedure

A multi-step procedure was used to raise adapted lines (Shah et al., 2002). Cell lines were subjected to an incremental increase of PEG and LiCl stresses.

The sequence of increasing polyethylene glycol (PEG) and LiCl concentrations were 5% PEG and 5 mM LiCl (5 passages), 10% PEG and 10 mM LiCl (10 passages), 15% PEG and 15 mM LiCl (15 passages) and 20% PEG and 20 mM LiCl for 25 passages. Concurrently, control lines were maintained in the absence of PEG and LiCl.

### Measurement of growth

Growth of cell suspension was estimated as relative growth rate (RGR) of sedimented cell volume (SCV) (Shah et al., 2002):

$$\text{RGR (week)}^{-1} = [\ln (\text{SCV}_{\text{final}}) - \ln (\text{SCV}_{\text{initial}})] / \text{weeks}$$

$$\text{INTOL} = \text{RGR (treatment)} / \text{mean RGR (respective media)}$$

The index of tolerance expresses the RGR for each treatment as a proportion to the mean RGR of the appropriate control.

## Extraction and determination of inorganic and organic cytosolutes

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

### Tolerance of cell lines to opposite stresses

The response of unadapted, polyethyleneglycol and LiCl adapted cell lines to ionic and osmotic stresses is presented in Figure 1a, which showed that the relative growth rates of osmotically (20% PEG) and ionically (20 mM LiCl) adapted cell lines were at par with the RGR of unadapted cell line in stress free medium and RGRs of all lines were also similar at their respective media (growth of unadapted line on stress free and PEG and LiCl adapted lines on medium supplemented with 20% PEG and 20 mM LiCl, respectively). On transfer to 20% PEG and 20 mM LiCl stresses, the RGRs of unadapted cells line decreased to 0.042 and 0.038, respectively. In contrast, the RGR of PEG adapted line slightly increased at LiCl stress, while RGR of LiCl adapted line slightly decreased at 20% PEG stress as compared to its growth on respective media. Statistical analyses showed highly significant ( $p < 0.005$ ) effects of stresses on growth with a significant interaction ( $p < 0.005$ ) between cell lines.

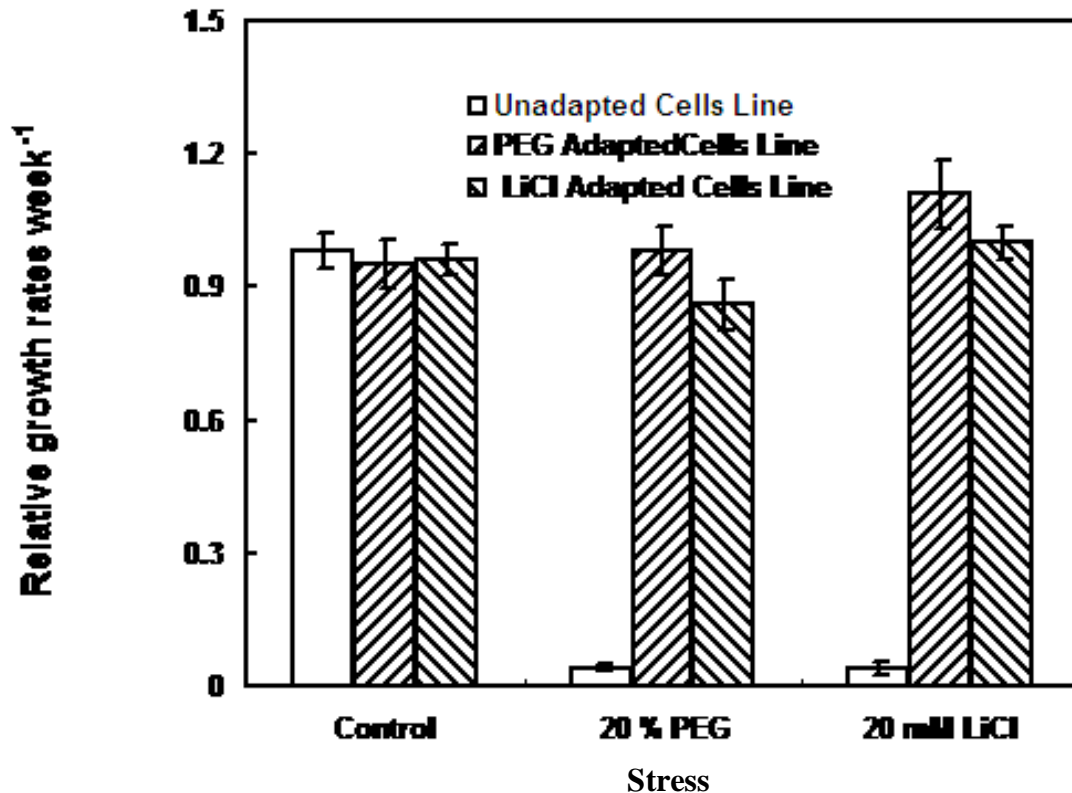
### Responses of adapted and unadapted lines to temperature stresses

#### Indices of tolerance

The indices of tolerance based on RGRs of adapted and unadapted cell lines grown at optimum temperature (27°C) were similar at their respective media. When these cell lines were exposed to low (18°C) and high (36°C) temperatures stresses, the growth of unadapted cell line completely ceased, while PEG and LiCl adapted cell lines were able to retain their tolerance (Figure 1b) The statistical analysis showed highly significant effect for stress and lines with significant interaction ( $p < 0.005$ ).

### Proline accumulation

Unadapted and LiCl adapted cell lines accumulated almost similar concentrations of proline on their respective media at optimum temperature (27°C), while PEG adapted lines had an order of magnitude enhanced level of proline at optimum temperature (Figure 2). When these lines were subjected to low (18°C) and high (36°C) stresses, the level of proline significantly increased at low and high temperatures, respectively. The analysis of



**Figure 1a.** Effect of polyethylene glycol and LiCl concentration on growth of unadapted ( $\square$ ), LiCl adapted ( $\boxtimes$ ) and polyethylene adapted ( $\boxplus$ ) cell lines of *O. sativa* L cv. Swat-1. Vertical bars represent the mean values of 5 replicates  $\pm$  standard error.

variance showed significant ( $p < 0.005$ ) effect for stress, lines and their interaction.

## DISCUSSION

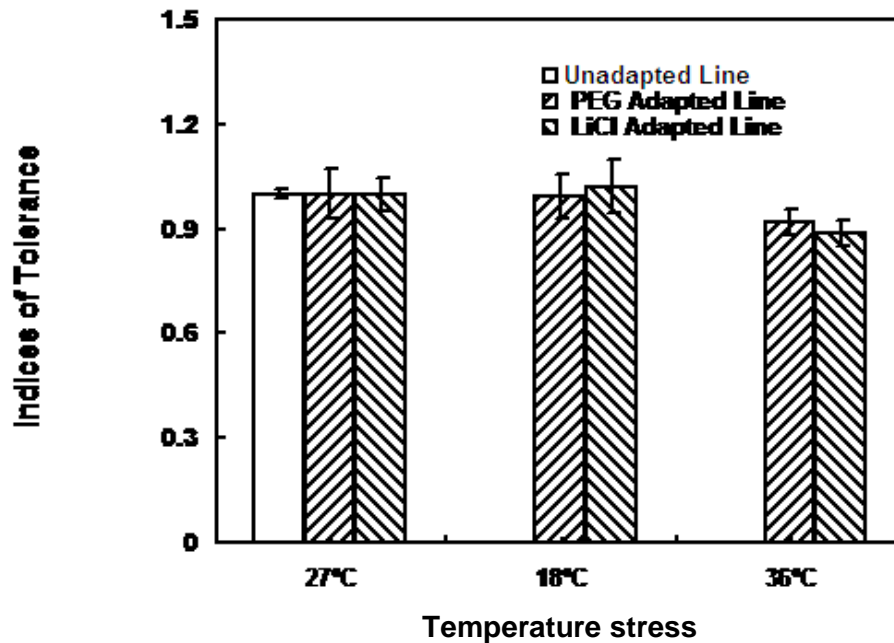
The present study aimed at investigating the phenomenon of cross adaptation in cell lines of a local rice cultivar Swat-1. The cell lines were developed by incremental increase in osmotic (20% polyethylene glycol) and ion specific (20 mM LiCl) stress agents, respectively.

Over all, the relative growth rates of adapted and unadapted cell lines were similar at their respective media (stress free medium for unadapted and 20% PEG and 20 mM LiCl for lines adapted to osmotic and ionic stresses, respectively), which indicated that addition of 20% PEG and 20 mM LiCl in the medium was no longer a stress for adapted cell lines (Figure 1a).

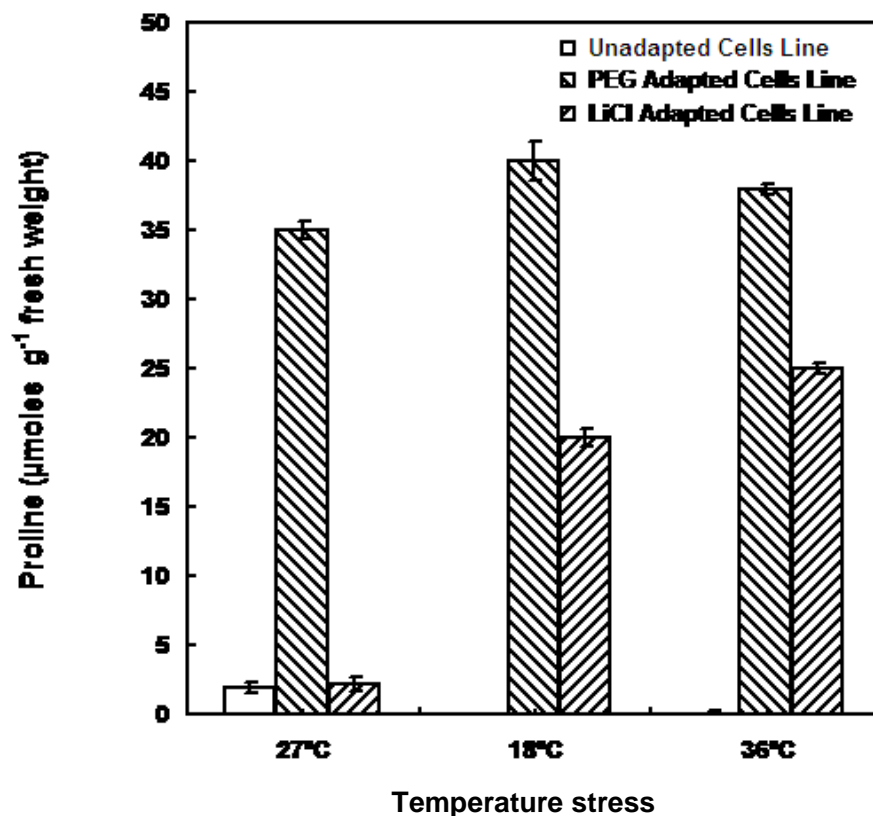
On the other hand, the parallel growth rates of adapted (55 passages on stress, that is, for 22 months) and unadapted cell lines on stress free medium showed that cell lines that adapted to osmotic and ion specific stresses were not at competitive disadvantage when grown back on stress free medium.

About 95% reduction in relative growth rate of

unadapted cells line was observed at osmotic (20% PEG) and ionic (20 mM LiCl) stresses, and complete cessation of growth was observed at low (18°C) and high (36°C) temperature stresses, respectively. By contrast, the adapted cell lines exhibited highly significant tolerance to either osmotic or ion specific or temperature (low and high) regimes, respectively (Figure 1a and b). The tolerance induced against one stress when it shows resistance to another stress is called cross tolerance (Takahashi et al., 1994). The occurrence of such type of tolerance is considered to be associated with the presence of common stressor in the selection environment. It is argued that common stressor affect normal cellular function in a similar way; as a result, plant cells make use of common pathways and components of stress entailing to cross tolerance response (Levitt, 1980; Pastori and Foyer, 2002; Alexieva et al., 2003). In this perspective, the cross tolerance of PEG adapted line to low (18°C) and high (36°C) temperature stresses (Figure 1a and b) could be as a result of the presence of common stressor (drought in this case) in the environment, because the basic physiological response of plant to drought, temperature and salt stress overlaps with one another, since all these stresses lead to dehydration an osmotic stress (Wang et al., 2003; Langridge et al., 2006;



**Figure 1b.** Effect of low and high temperature stress on indices of tolerance of unadapted ( $\square$ ), LiCl adapted ( $\boxtimes$ ) and polyethylene adapted ( $\boxplus$ ) cell lines of *O. sativa* L cv. Swat-1. Vertical bars represent the mean values of 5 replicates  $\pm$  standard error.



**Figure 2.** Effect of low and high temperature stress on proline accumulation of unadapted ( $\square$ ), LiCl adapted ( $\boxtimes$ ) and polyethylene adapted ( $\boxplus$ ) cell lines of *O. sativa* L cv. Swat-1. Vertical bars represent the mean values of 5 replicates  $\pm$  standard error.

Sreenivasulu et al., 2007). In our case, adaptation to PEG was apparently against drought stress, as adapted line accumulated about 17 folds more proline ( $35 \mu\text{mole g}^{-1}$  fresh weight) than unadapted cells line and the level remained stable under temperature stresses (Figure 2). The increased level of proline accumulation under drought and temperatures stresses show positive correlation with growth at the cellular and seedling stages (Shah et al., 2002; Song et al., 2005).

However, this explanation does not hold true while considering the response of LiCl adapted lines to osmotic and temperature stresses, respectively. Simply because LiCl adapted line did not share such common stressor with either PEG or temperature stresses because this line was adapted only to ionic stress that was neither present in osmotically induced nor in temperature (low and high) induced stresses. Further more, the tolerance to low temperature stress requires relatively higher proportion of un-saturated fatty acids in the plasma membrane, while for tolerance to high temperature stress, higher proportion of saturated fatty is required (Mahajan et al., 2005; Murakanmi et al., 2000). Therefore, the parallel tolerance of adapted lines to opposite stresses and to low and high temperature stresses is a tangible evidence revealing that presence of common stressor was not simply the reason for occurrence of cross tolerance. Rather it appeared like the consequence of priming or adaptation of soft wares for quick and effective signal perception and transduction which enables the cell to reprogram its response with respect to the altered stress. Therefore, this study helps in understanding the underlying mechanism(s) of cross tolerance in plants and the information generated provide the basis for devising crop improvement strategies for simultaneous breeding.

## ACKNOWLEDGEMENTS

This study was a part of Ph. D. program of the first author. It was supported in part by the grants from Higher Education Commission of Pakistan and Pakistan Science Foundation. The technical assistance of Mr. Sajjad Ahmad is gratefully acknowledged.

## REFERENCES

- Alexieva V, Ivanov S, Sergiene I, Karanov E (2003). Interaction between stresses. *BUIG. J. Plant Physiol. Special Issue.* 1-17.
- Bates LS, Waldren RP, Tear ID (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil.* 39: 205 - 206.
- Bowler C, Fluhr R (2000). The role calcium and activated oxygen as a signal for controlling cross tolerance. *Trends Plant Sci.* 5: 241- 246
- Chrispeels MJ, Sadava DE (2003). *Plants Genes and Crop Biotechnology.* American Society of Plant Biologists. Jones and Barlett (2<sup>nd</sup> eds). Inc. pp. 124-152.
- Fosket DE (1994). *Plant Growth and Development.* San Diego: Academic Press.
- Kreps JA, Wu YJ, Chang HS, Zhu T, Wang X, Harper JF (2002). Transcriptome changes for *Arabidopsis* in response to salt, osmotic and cold stress. *Plant Physiol.* 130: 2129-2141.
- Langridge P, Paltridge N, Fincher G (2006). Functional genomics and abiotic stress tolerance in cereals. *Func. Geno. Prot.* 4(4): 343-354.
- Levitt J (1980) *Responses of plants to environmental stresses.* Academic Press, New York.
- Mahajan S, Tuteja N (2005). Cold salinity and drought stress: An overview. *Arch. Biochem. Biophys.* 444: 139-154.
- Murakanmi Y, Tsuyama M, Kobayashi Y, Kodama H, Iba K (2000). Trienoic fatty acids and plant tolerance of high temperature stress. *Sci.* 287: 476-479.
- Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Plant Physiol.* 15: 473-497.
- Pastori GM, Foyer CH (2000). Common components, net works, and pathways of cross tolerance to stress. The central role of redox and abscisic acid mediated controls. *Plant Physiol.* 122: 667-676
- Ryu SB, Costa A, Xin Z, Li PH (1995). Induction of cold hardiness by salt stress involves synthesis of cold and abscisic acid-responsive proteins in potato (*Solanum commersonii* Dunn). *Plant Cell Physiol.* 36: 1245-1251.
- Serrano R, Cullianz- Macia F, Moreno V (1999). Genetic engineering of salt and drought tolerance with yeast regulatory genes. *Hort. Sci.* 78: 261-269.
- Shah SH, Tobito S, Shano M (2002). Cation cotolerance phenomenon in cell cultures of *Oryza sativa* adapted to LiCl and NaCl. *Plant cell, Tissue and Org. Cul.* 71: 95-101.
- Song SQ, Lei YB, Tian XR (2005). Proline metabolism and cross tolerance to salinity and heat stress in germinating wheat seed. *Russ. J. Plant Physiol.* 52(6): 897-904.
- Sreenivasulu N, Sporoy SK, Kishor PBK (2007). Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. *Gene,* 188: 1-13.
- Takahashi R, Josheer N, Kitagawa Y (1994). Induction of chilling resistance by winter stress and cDNA sequence analysis and expression of water stress-geulated genes in rice. *Plant Mol. Biol.* 26: 339-352.
- Wahid A, Gelani S, Ashraf M, Foolad MR (2007). Heat tolerance in plants: An overview. *Environ. Expt. Bot.* 61: 199-223.
- Wang W, Vinocur B, Altman A (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta,* 218: 1-14.