

Proline, sodium and potassium concentration changes in gamma rays and NaCl treated potato calli

Behrokh Shojaie¹, Ali Akbar Ehsanpour^{1*} and Mohammad Reza Abdi²

¹Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

²Department of Physics, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Received 18 November 2010

Accepted 18 December 2010

Abstract

In this study, the effects of gamma rays on changes of proline, sodium and potassium in potato calli (*Solanum tuberosum* L.) cultivar "White Desiree" were investigated. After irradiation of calli by 60 and 80 Gy of gamma radiation, irradiated and non irradiated calli were transferred to a callus production medium containing 0, 30, 60, 90, 120 and 150 mM NaCl. After 26 days post treatment amounts of proline, Na⁺ and K⁺ were measured. The results showed that generally in each of gamma ray treatment, amounts of proline at 120 and 150 mM NaCl were significantly more than 0, 30, 60 and 90 mM salt. A significant difference observed only at 150 mM NaCl between irradiated and non irradiated calli. This result could be related to the proline role in osmotic adjustment at high concentration of salt. The amounts of sodium increased by increasing salt the concentration but the amount of potassium decreased. These results showed that the sodium influx inhibited the potassium uptake, and may be because of strong inhibitory effect of sodium on the potassium uptake system or efflux potassium from the cells. The Na⁺/K⁺ ratio decreased at 150 mM by 60 and 80 Gy of gamma radiation.

Keywords: callus, gamma ray, proline, potato

Introduction

Salt stress is the major limiting factor for plant growth and agricultural productivity (Ashraf and Harris, 2004). Salt stress induces other stresses such as ionic (Zhu, 2001b), osmotic (Xiong and Zhu, 2002a), nutrient disorder and oxidative stresses (Mansour et al., 2005). Plants have several strategies against these stresses. For example, avoidance of ionic toxicity and ion exclusion, salt compartmentalization and control of long distant transport, synthesis of osmolites and antioxidant enzyme, gene regulation and phytohormonic responses (Yeo, 1998; Ashraf, 2004; Ashraf and Foolad, 2007). These strategies act for establishment of ion homeostasis, detoxification and plant growth control (Zhu, 2001b). Plants are divided to halophyte and glycophyte (Flowers and Flowers, 2005). Potato as a glycophyte plant is sensitive to salt stress and studies showed that damage thresholds of tetraploid potatoes are from 15 to 30 mM NaCl (Shaterian et al., 2005).

Under salt stress, high concentrations of sodium disturb potassium and other essential ions uptake (Shi et al., 2002). Potassium is vital for the maintenance of cell turgor and the activity of many

enzymes (Fu and Luan, 1998). It has also a main effect on the membrane potential and cell physiology (Spalding et al., 1999). Under the NaCl stress, intracellular Na⁺ concentration is increased and K⁺ concentration is decreased (Horie and Schroeder, 2004). Plants regulate Na⁺ efflux and accumulation of essential ions by various transporters in order to maintain high K⁺/Na⁺ ratio in the cytosol (Niu et al., 1995; Shi et al., 2002; Zhu, 2002; Shi and Zhu, 2002; Ashraf, 2004).

The synthesis and accumulation of compatible solute or/and osmolytes are the main mechanisms of adaptation to salinity especially in glycophytes (Flowers and Flowers, 2005; Benlloch-Gonzalez, 2005). Compatible solutes include proline, sucrose, trehalose, glycinebetaine, alaninebetaine, prolinebetaine, choline O-sulfate, hydroxyprolinebetaine, piperolatebetaines, glycerol, mannitol, sorbitol, pinitol, ornitol fructans cause decreasing salt stress. In plant cells, synthesis and accumulation of compatible solutes increase in response to salt and other abiotic stresses, for cellular osmotic adjustment, detoxification of reactive oxygen species and scavenging free radicals, protection of membranes, proteins, enzymes and other cellular structures (Nanjo et al., 1999; Zhu, 2001b; Ashraf and Harris, 2004; Ashraf and Foolad, 2007). Up regulation of *P5CS* gene is a good example which is expressed by salt or drought

*Corresponding author E-mail:
ehsanpou@yahoo.com

stresses (Yoshiba et al., 1999).

Ionizing radiation such as gamma rays may induce mutations in plant cells. Nowadays, gamma rays is being used widely in plant biology to induce desirable changes such as production of salt tolerant cell lines (Baek et al., 2005), alteration of shape and color of flowers (Misra et al., 2003; Buiatti and Ragazzini, 1965), increasing production of secondary metabolites (Chung et al., 2006), modulation of quantitative and qualitative characteristics in crops (Maity et al., 2005).

The aim of this study was to evaluate the effect of gama radiation on salt tolerance of potato callus (*Solanum tuberosum* L.) c.v. White Desiree using measurement of some physiological parameters.

Materials and Methods

For the callus production, leaf segments from *in vitro* grown potato (*Solanum tuberosum* L.) cultivar White Desiree were cultured on Murashig and Skoog, medium (1962) containing NAA, 2,4-D and Kinetin, 2mg/l each, yeast extract (1g/l), sucrose 3% (w/v) and agar 0.8% (w/v). The pH was adjusted at 5.8. All samples were grown in the culture room and were kept in the dark at 25±2°C. After 4 to 5 weeks callus was initiated from leaf explants. Calli were sub-cultured with two weeks interval in the same medium. After the first sub-culture calli were gamma irradiated using dosage of 60 and 80 Gy (Atomic energy organization of Isfahan). Then, they were transferred to the previous medium containing 0, 30, 60, 90, 120 and 150 mM NaCl. After 26 days the post treatment amounts of proline, sodium and potassium were measured. The modified Bates (1973) method was used for proline determination. In this method, callus (40 mg) was homogenized in 1.7 ml of 3% (w/v) aqueous sulfosalicylic acid. Extractions were transferred to centrifuge tubes and centrifuged at 14,000g for 20 min, 1ml of each supernatant

transferred into a 10 ml test tube, then 1ml of glacial acetic acid and 1ml of the ninhydrin reagent added to each tube and heated for 1 h at 100°C. Tubes were then cooled using cold water and 2 ml of toluene added to each tube, sealed with aluminum foil and vortexed at 30 rpm for 2 min. The phase separation was completed after 30 min and the upper phase was used for measuring the absorbance at 520 and 490 nm. The standard curve solutions of proline were prepared using 1.7 ml of 3% (w/v) sulfosalicylic acid at a concentration of 5 to 200 µM (0.0006 to 0.0230 mg/ml) (Ringle et al., 2003).

Flame photometry method was used for measuring Sodium and potassium content of the treated calli based on the method described by Skoog et al., (2007). First, calli were collected and dried at 60°C for at least 5 days, 0.01 g of each powder of dried callus was digested with 10 ml 3 % (w/v) aqueous sulfosalicylic acid for 24 h at 4°C, sample extract purified with watman No. 1 filter paper and Na⁺ and K⁺ concentrations were measured.

All experiments were carried out in five replications. Data were analyzed by Sigma state version 2.8 and ANOVA and the mean differences were compared by Duncan test at P<0.05.

Results

The general pattern of proline in irradiated and non irradiated calli was relatively increased as salt concentration increased (figure 1). At 150 mM NaCl, the amounts of proline in irradiated calli with 60 and 80 Gy were less than those of non irradiated calli and the difference between the irradiated and non irradiated calli was significant (P<0.05). The proline content of the irradiated calli using 60 and 80 Gy was not significant. However, the highest amount of proline was observed at 150 mM NaCl in non irradiated calli.

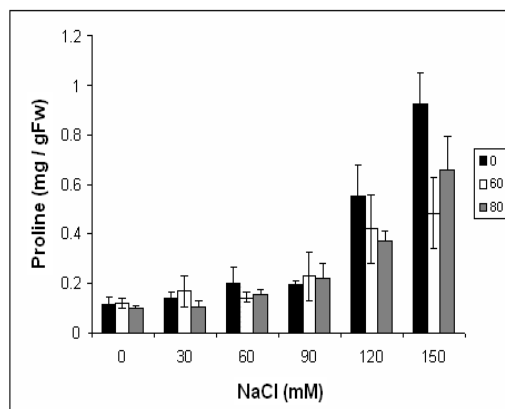


Figure 1. Proline content of treated potato calli with gamma rays and NaCl (Data are means of five replication ± Std).

In order to detect the real increasing in the amount of proline with increasing salt concentration and because of the difference between the spectrum of reaction product of proline and ninhydrin and the spectra of reaction products of interfering substances and ninhydrin, we measured the absorbance of proline at 490 and 520

nm by spectrophotometer based on the method of Ringle and colleagues (2003). For the net proline content, this ratio should be between 0.82 and 0.88. However, the A490/A520 ratio of the callus extracts were more than 0.9 in concentrations up to 60 mM NaCl.

Table 1. Means of A490/A520 ratio in treated potato calli with gamma rays (0, 60 and 80 Gy) and NaCl (0, 30, 60, 90, 120 and 150 mM).

Treatment gamma rays and NaCl	A490/A520 ratio
0 and 0	0.93
0 and 30	0.94
0 and 60	0.92
0 and 90	0.88
0 and 120	0.85
0 and 150	0.88
60 and 0	0.96
60 and 30	0.91
60 and 60	0.94
60 and 90	0.87
60 and 120	0.84
60 and 150	0.89
80 and 0	0.94
80 and 30	0.95
80 and 60	0.92
80 and 90	0.88
80 and 120	0.86
80 and 150	0.85

With increasing salt concentration, the Na⁺ content in irradiated calli showed an increasing pattern similar to that in non irradiated calli (figure 2).

In non irradiated calli, amounts of Na⁺ in salt free medium and medium with 30 mM NaCl showed a significant difference ($p < 0.05$), compared to the other salt concentrations. However, there was a significant difference ($p < 0.05$) between 0 and 30 mM NaCl.

In irradiated calli with 60 Gy of gamma ray, the

amount of sodium at concentrations of 90, 120 and 150 mM NaCl was higher than that of 0 and 30 mM NaCl. A significant difference in Na⁺ content between 60 mM treatments of NaCl with 0 and 90 mM treatments was observed. In irradiated calli with 80 Gy, the result was similar to non irradiated calli, but no significant difference between treatments of 0 and 30 mM NaCl was observed. There was also a significant difference ($p < 0.05$) between non irradiated and irradiated calli by using 60 Gy of gamma ray at 90 mM NaCl concentration.

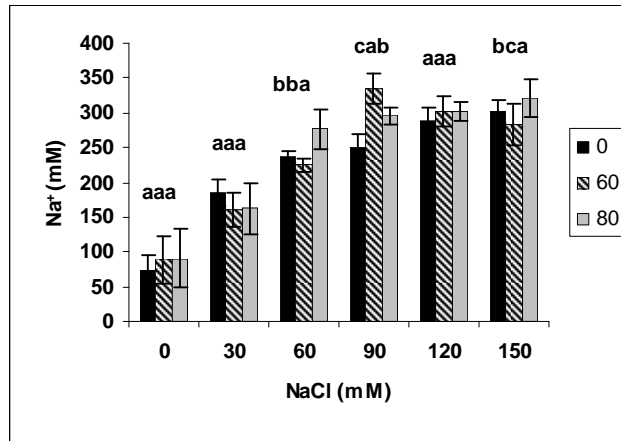


Figure 2. Amounts of sodium in treated potato calli with gamma rays and NaCl (Data are the means of five replication \pm SD).

As shown in figure 3, in non irradiated calli the amount of K⁺ at 0 mM NaCl was higher than other salt concentrations (30, 60, 90, 120 and 150 mM) significantly ($p < 0.05$). In 60 Gy irradiated calli, similar to non irradiated calli, there was a significant difference between 0 mM NaCl and other concentrations. The amount of potassium at 120 mM NaCl was significantly less than 30, 60 and 90 mM ($p < 0.05$). In irradiated calli with 80 Gy,

there was not a significant difference among various salt concentrations.

In salt free media, there was a significant difference between non irradiated and irradiated calli ($p < 0.05$). Also at 60 mM NaCl, a significant difference was observed ($p < 0.05$) between irradiated, with 60 Gy, and non irradiated calli as well as a significant difference between both gamma ray dosages.

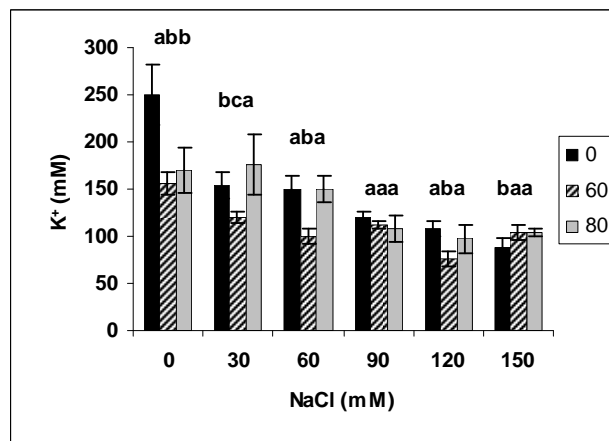


Figure 3. Amounts of potassium in treated calli of potato with gamma rays and NaCl (data are the means of five replication \pm SD).

We also analyzed Na⁺/K⁺ ratio. As shown in figure 4, generally in all radiation treatments, Na⁺ / K⁺ ratio increased with increasing of salt concentrations. In non irradiated calli Na⁺ / K⁺ ratio at 150 mM NaCl was more than other concentrations. In 60 Gy irradiated calli the ratio was increased up to 120 mM NaCl but at 150 mM

NaCl significantly. In 80 Gy the irradiated calli, Na⁺/K⁺ ratio increased up to 90 mM NaCl however, this ratio from 90 to 150 mM NaCl showed no significant difference. In addition, at 60, 90, 120 and 150 mM NaCl a significant difference between 60 Gy irradiated and non irradiated calli was recorded.

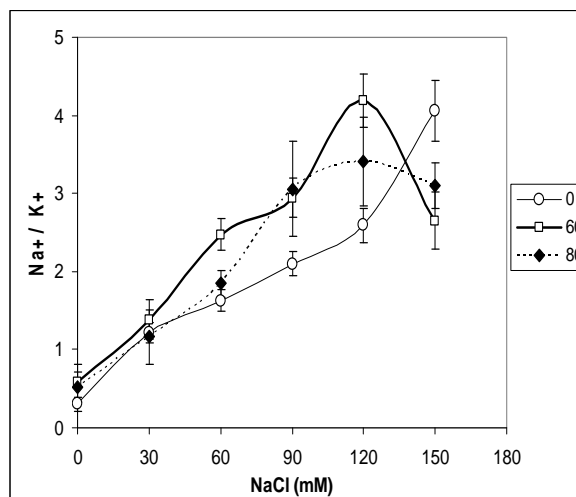


Figure 4. The Na^+/K^+ ratio in treated calli of potato with gamma rays and NaCl (data are the means of five replication \pm SE).

Discussion

The results of proline assessment showed a general increasing of proline content at high salt concentrations. Therefore, in c.v White Desiree potato calli, proline may play a main role in cellular osmotic adjustment. This result is similar to other reports such as the increasing proline in callus of *Medicago sativa* with increasing of salt concentrations (Ehsanpour and Fatahian, 2003).

When proline was measured at A490/A520 nm, the means of A490/A520 ratio for proline in irradiated and non irradiated calli treated with NaCl up to 60 mM were more than 0.9, and the ratios for 90 to 150 mM NaCl were between 0.82 and 0.88 respectively. It seems that up to 60 mM NaCl, the proline content increased and at A520. The observed data was not only related to proline but also related to other interfering materials such as sugars or other amino acids (except proline) that react with the reagent ninhydrin. Consequently, the products have a greater absorbance at 490 nm than at 520 nm. However, these ratios indicate that at 90, 120 and 150 mM NaCl actual content of proline has increased (Ringle et al., 2003). In another word, In 60 Gy irradiated calli, it is likely that salt stress increased the proline content but the ratio of 0.9 shows that at least some of the interactions inside the cells are not due to the proline accumulation, but at 90, 120 and 150 mM NaCl, the irradiated calli showed a ratio between 0.82 to 0.88 which indicates real proline content. It means that at high concentration of salt, the accumulation of proline grows high. The accumulation of proline inside the cell possibly inhibits the other interaction with ninhydrin which remains to be.

In non irradiated calli treated with, up to 90 mM

NaCl, the amount of proline did not increase, but at 120 and 150 mM NaCl increasing of proline was significant. This result apparently indicates that concentrations of up to 90 mM NaCl, cells were not affected by osmotic stress and therefore, cells did not accumulate more proline. The other reason for such effect may be the increasing of other osmolytes such as sugar. This idea could be confirmed by the results of the A490/A520 ratio for proline in the treated calli.

There is no significant difference in proline level of treated calli between 120 and 150 mM NaCl, it seems that the effects of osmotic shock at 120 and 150 mM NaCl were similar and it is possible that at 150 mM NaCl these cells, in addition to proline, had synthesized and accumulated other compatible solutes. The other possible reason may be the random mutation in their genome. Our results of the study on DNA breaks in gamma irradiated potato has already confirmed the possible occurrence of the random mutation in potato callus (Shojaie et al., 2009). Other studies have also shown that gamma rays can induce mutations in plant cells (Nagata et al., 1999). Thus, it is likely that gamma ray with dosage of 60 Gy could induce random mutation in calli and therefore, these calli on medium containing 150 mM NaCl were not able to synthesize more proline. This could be confirmed by comparison of 60 Gy irradiated calli at 150 mM NaCl with non irradiated calli at the same salt concentration.

Irradiated, with 80 Gy, and non irradiated calli showed similar responses. It seems that irradiated calli treated with 80 Gy at 90 mM NaCl were more sensitive to salt stress than non irradiated calli. It is possible that concentration of 90 mM NaCl could induce osmotic stress in these cells. Therefore, at

90 mM NaCl, cells increased proline synthesis to prepare for salt tolerance. It is suggested that proline have a protective role in cells when they are faced to the osmotic stress (Nanjo et al., 1999).

Since in irradiated calli with 60 and 80 Gy, the amounts of proline at the highest salt concentration were less than non irradiated calli, it is possible that irradiated calli have suffered less than non irradiated calli at 150 mM NaCl. Increasing of proline is a defense response of the cell to damages (Delauney and Verma, 1993; Liu and Zhu, 1997). The possible reason for the difference between irradiated calli and non irradiated calli at 150 mM NaCl may be due to an increase in anti oxidant enzymes activity (Azooz et al., 2009).

The results of Na⁺ and K⁺ content in the examined calli showed that generally, the amount of sodium increased with increasing salt concentration but, the amount of potassium decreased (in the calli received 80 Gy irradiation). The results could be due to Na⁺ influx to the cells and K⁺ extrusion with increasing salt concentration. In 80 Gy irradiated calli, at all salt concentrations, the amount of K⁺ with increasing salt concentration showed no difference but, their amount of Na⁺ increased. This might be related to the accumulation of Na⁺ in vacuoles (Horie and Schroeder, 2004). Therefore K⁺ influx was not inhibited or K⁺ exclusion was not induced. Thus, the amount of K⁺ in calli on media containing salt was similar to the salt free medium.

However, despite of high Na⁺ compared to K⁺ content in the calli, treated with NaCl, it was notable that the gamma irradiation changed the ratio of Na⁺/K⁺ in the calli. This ratio increased up to 120 mM NaCl (in irradiated calli), but at 150 mM NaCl, it was decreased dramatically; it might be due to the overall reduction of plant growth at high concentration of NaCl (figure 4). Since the ratio of Na⁺/K⁺ is critical for salt tolerance, decreasing this ratio at high concentration of salt (150 mM) by 60 and 80 Gy of gamma radiation is promising for the future research.

The most notable conclusion of this study is the specific effects of gamma radiation on proline biosynthesis pathway or proline feed back inhibitory system of potato cultivar White Desiree. It should be kept in mind that in plants increasing the salt concentration induce proline synthesis while, in the present study opposite results were obtained in irradiated calli. In addition, the gamma radiation affected Na⁺ and K⁺ content as well as the Na⁺/K⁺ ratio. These findings might be due to genetic or epigenetic changes of callus cells. However, we can conclude that gamma radiation with, dosages of 60 and 80 Gy, may induce positive

effect on salt tolerance in potato calli c.v. white Desiree but more precise researchs are needed for selection of salt tolerant cell line in potato callus.

Acknowledgment

Authors wish to thank all members of the Graduate Council of University of Isfahan for their support.

References

- 1- Ashraf M. (2004) Some important physiological selection criteria for salt tolerance in plants. *Flora* 199: 361–376.
- 2- Ashraf M. and Foolad M. R. (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany* 59: 206–216.
- 3- Ashraf M. and Harris P. J. C. (2004) Potential biochemical indicators of salinity tolerance in plants. *Plant Science* 166: 3-16.
- 4- Azooz M. M., Ismail A. M. and Abou Elhamd M. F. (2009) Growth, Lipid Peroxidation and Antioxidant Enzyme Activities as a Selection Criterion for the Salt Tolerance of Maize Cultivars Grown under Salinity Stress. *International Journal of Agriculture and Biology* 11: 21-26.
- 5- Baek M. H., Kim J. H., Chung B. Y., Kim S. J. and Lee I. S. (2005) Alleviation of salt stress by low dose γ irradiation in rice. *Biologia Plantarum* 49: 273-276.
- 6- Benlloch-Gonzalez M., Fournier J. M., Ramos J. and Benlloch M. (2005) Strategies underlying salt tolerance in halophytes are present in *Cynara cardunculus*. *Plant Science* 168: 653-659.
- 7- Buiatti M. and Ragazzini R. (1965) Gamma-ray induced changes in the carnation, *Dianthus caryophyllus* L. *Radiation Botany* 5: 99-105.
- 8- Chung B. Y., Lee Y. B., Baek M. H., Kim J. H., Wi S. G. and Kim J. S. (2006) Effects of low-dose gamma-irradiation on production of shikonin derivatives in callus cultures of *Lithospermum erythrorhizon* S. *Radiation Physics and Chemistry* 75: 1018–1023.
- 9- Delauney A. and Verma D. P. S. (1993) Proline biosynthesis and osmoregulation in plants. *The Plant Journal* 4: 215–223.
- 10- Ehsanpour A. A. and Fatahian N. (2003) Effects of salt and proline on *Medicago sativa* callus. *Plant cell, Tissue and Organ Culture* 73: 53-56.
- 11- Flowers T. J. and Flowers S. A. (2005) Why does salinity pose such a difficult problem for plant breeders. *Agricultural Water Management* 78: 15-24.
- 12- Fu H. H. and Luan S. (1998) AtKUP1: a dual-affinity K⁺ transporter from *Arabidopsis*. *The Plant Cell* 10: 63–74.
- 13- Horie T. and Schroeder J. I. (2004) Sodium transporters in plants, diverse genes and physiological functions. *Plant Physiology* 136: 2457–2462.
- 14- Liu J. and Zhu J. K. (1997) Proline accumulation and

- salt-stress-induced gene expression in a salt-hypersensitive mutant of *Arabidopsis*. *Plant Physiology* 114: 591–596.
- 15- Maity J. P., Mishra D., Chakraborty A., Saha A., Santra S. C. and Chanda S. (2005) Modulation of some quantitative and qualitative characteristics in rice (*Oryza sativa* L.) and mung (*Phaseolus mungo* L.) by ionizing radiation. *Radiation Physics and Chemistry* 74: 391–394.
 - 16- Mansour M. M. F., Salama K. H. A., Ali F. Z. M. and Abou Hadid A. F. (2005) Cell and plant responses to NaCl in *Zea mays* L. cultivars differing in salt tolerance. *General and Applied Plant Physiology* 31: 29-41.
 - 17- Misra P., Datta S. K. and Chakrabarty D. (2003) Mutation in flower colour and shape of *Chrysanthemum morifolium* induced by γ radiation. *Biologia Plantarum* 47: 153-156.
 - 18- Nagata T., Todoriki S., Hayashi T., Shibata Y., Mori M., Kanegae H. and Kikuchi S. (1999) γ Radiation induces leaf trichome formation in *Arabidopsis*. *Plant Physiology* 120: 113-119.
 - 20- Nanjo T., Kobayashi M., Yoshiba Y., Kakubari Y., Yamaguchi-Shinozaki K. and Shinozaki K. (1999) Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Letters* 461: 205–210.
 - 21- Niu X., Bressan R. A., Hasegawa P. M. and Pardo J. M. (1995) Ion homeostasis in NaCl stress environment. *Plant Physiology* 109: 735–742.
 - 22- Ringel C., Siebert S. and Wienhaus O. (2003) Photometric determination of proline in quartz microplates: remarks on specificity. *Analytical Biochemistry* 313: 167-169.
 - 23- Shaterian J., Waterer D., Jong De H. and Tanino K. K. (2005) Differential stress responses to NaCl salt application in early- and late-maturing diploid potato (*Solanum* sp.) clones. *Environmental and Experimental Botany* 54: 202–212.
 - 24- Shi H. and Zhu J. K. (2002) Regulation of the vacuolar Na⁺/H⁺ antiporter gene AtNHX1 expression by salt stress and abscisic acid. *Plant Molecular Biology* 50: 543-550.
 - 25- Shi H., Quintero F. J., Pardo J. M. and Zhu J. K. (2002) The putative plasma membrane Na⁺/H⁺ antiporter SOS1 controls long distance Na⁺ transport in plants. *The Plant Cell* 14: 465–477.
 - 26- Shojaie B., Ehsanpour A. A. and Abdi M. R. (2009) Detection of double and single strand DNA breaks in gamma irradiated potato callus using comet assay. *Malaysian Applied Biology* 38: 1-4.
 - 27- Skoog D. A., West D. M., Holler F. J. and Crouch S. R. (2007) *Analytical Chemistry: An Introduction*, New Age International PVT, UK 594-631 pp.
 - 28- Spalding E. P., Hirsch R. E., Lewis D. R., Qi Z., Sussman M. R. and Lewis B. D. (1999) Potassium uptake supporting plant growth in the absence of AKT1 channel activity, inhibition by ammonium and stimulation by sodium. *The Journal of General Physiology* 113: 909-918.
 - 29- Xiong L. and Zhu J. K. (2002a) Salt tolerance. *The Arabidopsis Book*, Published by: American Society of Plant Biologists, USA, 24 pp.
 - 30- Yeo A. (1998) Molecular biology of salt tolerance in the context of whole-plant physiology. *Journal of Experimental Botany* 49: 915-929.
 - 31- Yoshiba Y., Nanjo T., Miura S., Yamaguchi-Shinozaki K. and Shinozaki K. (1999) Stress-responsive and developmental regulation of D1-pyrroline-5-carboxylate synthetase 1 (P5CS1) gene expression in *Arabidopsis thaliana*. *Biochemical and Biophysical Research Communications* 261: 766–772.
 - 32- Zhu J. K. (2001a) Plant salt tolerance. *Trends in Plant Science*, 6: 66-71.
 - 33- Zhu J. K. (2001b) Cell signaling under salt, water and cold stresses. *Current Opinion in Plant Biology* 4: 401-406.
 - 34- Zhu J. K. (2002) Salt and drought stress signal transduction in plants. *Annual Review of plant biology* 53: 247-273.