Effected of various osmotic stress in Rice callus and prolin accumulation.

تاثير الشد الازموزي على نمو كالس الرز وتجمع البرولين

Hussein Sami Nagi
Kufa University
College of Education

Ali Yasser Hafith
Kufa University
Collage of Education

Abstract:

Calls obtained from two rice cultivars (Anber 33 and IPA1) were Exposed to different osmotic stress intensities followed by a period of stress relief. Relative growth rate , callus water content and changes in organic and inorganic solutes were determined at the end of stress. After the stress period callus derived from both cultivars showed a decrease in relative growth rate, but at lesser exent in Anber 33 than IPA1 cultivar. Same tendency was recorded in the callus water content under mannitol induced osmotic stress.The in Organic solute seemed to have no contribution in the osmotic adjustment in mannitol-stressed calli since k^+ and Ca^{2+} concentrations decreased drastically while Na^+ and Mg^{2+} concentrations were not affected. The accumulation of proline occurred in both cultivars and was more marked in IPA1 than Anber 33 cultivar. At the end of the relief period, we observed that all the considered Parameters have recovered completely to reach the control levels. According to these results, we conclude that the drought stress-induced changes are reversible, at the least at the cellular level, in Oryza Sativa L. cultivars.

المستخلص :

تم في هذه التجربه دراسه تاثير الشد الازموزي وتجمع البرولين على نمو كالس صنفين من اصناف الرز هما عنبر 33 واباء 1 حيث عرض كالس هذه الاصناف الى جهود ازموزيه مختلفه ولوحظ بعدها انخفاض في معدل النمو النسبي ولكن هذا الانخفاض حدث بصوره اقل في في الصنف عنبر 33 مقارنه بالصنف اباء 1 . وقد سجلت نفس النتيجه في محتوى الكالس عند تعرضه الى نفس الظروف من الشد الاوزموزي , اما بالنسبه للمواد الغير عضويه الذائبه فقد اظهرت التجربه بان ليس لـه دور في تنظيم الظغط الاوزموزي . وجد ان تركيز البوتاسيوم والكالسيوم قد انخفض تحت تاثير الشد او الضغط الاوزموزي بينما لم يتاثر تركيز الصوديوم والمغنيسيوم . كما تبين من التجربة ان الحامض الاميني البرولين قد تجمع في كلا الصنفين وكان تجمعة اكثر وضوحا في الصنف اباء 1 مقارنة بالصنف عنبر 33 .

Introduction:

Drought stress remains an ever-growing problem that severely limits crop production world wide and causes important agricultural losses particularly in arid and semi-arid areas (Boyer, 1982). Drought induced osmotic stress triggers a wide range of perturbation ranging from growth and water status disruption to the modification of ion transport and uptake systems (Bajji et al ., 2000) . Upon exposure to water deficit, plants exhibit physiological, biochemical and molecular responses at both the cellular and whole plant levels (Hasegawa, 2000).

Generally, the plants accumulate some kinds of organic and inorganic solutes in the cytosol to raise osmotic pressure and the driving gradient for water uptake (Rhodes and Samaras, 1994). Among thes solutes, praline is the most widely studied (Delauney and Verma, 1993).
It have been suggested that the increase of free proline levels is a symptom of injury that results from imbalances in other pathways (Bhaskaran et at., 1985). Also, the beneficial roles of prolin in conterring osmotolerance have been widely reported (Kavikishor et at., 1995).

Tissues cultures system is useful for the evalution of tolerance to environmental stress because the stress conditions can be easily controlled in vitro. Moreover, in vitro culture provides a uniform population synchronously developing plant cells without involving regulatory mechanisms that naturally repaired at the whole plant level (Tal, 1983).

The present work was therefore performed in sight to gain information on the processes taking place at the cellular level in rice (Oryza satival) after exposure to various osmotic stress intensities and its subsequent relief in relation with relative growth rate, callus water content, ion uptake and proline accum

Material and Methods

1- Plant material:

In this study, used two Oryza satival cultivars (Anber 33 and IPA1). Callus tissues initiation and growth frome epicotyl. Rice seeds disinfected with 70% ethanol for 0.5 minit and 3% $NaOcl_2$ for 5mn after that the seeds disinfected by distilled water. Seeds cultural in tubes (50mg) content 15ml (Ms) medium in rate 4 seeds/ tube the tubes in cubated in growth incubater upon 2±25c^o for to days. Used epicotyl for culturing initiation.

2- Cultre condition:

Callus induction was carried out as described by (Gandonou et at., 2005). After 6 weeks, calli were weighed and trans ferred to the same M.S Medium (Murashige and Skoog; 1962), (absence of stress and osmotic pressure of 0.4 Mpa). M.s medium added with mannitol in order to obtain final osmotic pressures of about 0.62 Mpa, 0.84 Mpa and 1.08 Mpa.

Callus cultures were incubated in the same conditions as describe above. After one month of stress, calli were divided in two set. One set was weighed and further characterized for solutes accumulation. The second set was transferred to Ms medium without mannitol for a subsequent period of one month.

For each period (stress and recovery), the callus relative growth rate (R.G.R) was expressed according to the formula:

s

The callus water content was calculated as:

$$\frac{(Fresh\ weight\ - dry\ weight\ )}{dry\ weight}$$

3- Determination of praline concentration*:*

Proline was extracted as described by (Tal, 1983). 200mg of callus fresh matter was homogenized in 4ml of methanol-choloroform-water at 4c^o and then was centrifuged at 20,000 X g for 30min. Super natants were then incubated at 4c^o for 12h in presence of 0.25 ml chloroform and 0.9ml distill water. Prolin was quantified in the upper-phase using ninhydrin acid reagent according (Bares et al., 1973). The chromophore containing was extracted in 4ml of toluene and measured spectro photometer at 520nm.

4- Determination of ion concentration:

For ion measurement, callus were first rinsed for 5min with cool distilled water and ther were then oven-dried at 80c^o for 72h and grounded. The dry matter obtained was used for mineral analysis. The major cation were extracted after digestion of dry matter with HNO3 acid. The extract was filtered prior to analysis. Na^+ and K^+ concentrations were determined using spectrophotometer. Ca^{2+} and Mg^{2+} concentrations were quantified by atomic absorption spectrophotometer.

5- Statistical analysis

All the measurement were repeated on two sets of 30 to 40 calli and showed similar results. Data present therefore are obtained from on single experiment. Each value is presented in the form of mean ± standard error with a reading of at least four samples per treatment. The analysis of the main effects of the stress intensity as well as the recovery data was based on the analysis of variance. All statistical analysis were preformed using SAS program (SAS Institute, 1992).

Results and discussion*:*

Relative growth rate (R.G.R.) appeared to be remarkably influenced by genotype, since a significant difference among cultivars was recorded even in the absence of stress. R.G.R. was higher in (Anber 33) an lower in (IPA1) (Figur1) Manitol induced osmotic stress im paired significant (R.G.R.) in both cultivars (F= 349.45, P<0.001) but at lesser extent in Anber 33 than IPA1 (Figure 1).

Thus at the osmotic pressure of 0.62 Mpa, (R.G.R.) decreased to approximately 29% and 34% in (Anber 33) and (IPA1) respectively.

The callus water content decreased significantly (F=262.89, P<0.001) and proportionally to the stress intensity in the medium, in similar manner to the (R.G.R) (Figure2). Thus, at the highest osmotic pressure, water callus content declined to about 45% and 48% of the control in (Anber 33) and (IPA1). Respectively, these findings indicated the important loss of water and they are in agreement with those reported in several other species such as Triticum duram (Bajj et al., 2000 ; Lutts et al., 2004), Oryza sativa (Lutts et al., 1996). Growth inhibition under osmotic conditions might be mainly due to the reduction in cytoplasmic volume and the loss of cel turgor as result of osmotic outflow of intracellular water (Rhodes and Samara, 1994).

Among the cultivars, our results suggested that (Anber 33) was slightly more drought resistant than (IPA1) at the cellular level and that the drought resistance is closely related to the maintenance of an elevated water status under such conditions. No significant difference was recorded after the stress relief for the (R.G.R.) and callus water content parameters. Thus callus relative growth resumed once and both (Anber 33 and IPA1) calli recovered growth to approximately 100% at all the stress intensities as shown in (Figure1). Similary the water status of both cultivars increased until reaching control levels at the end of the recovery period irrespective of the applied stress intensity (Figure2). These result also indicated that the viability was maintained during the stress treatment as was reported in wheat calli (Bajji et al., 2000; Al-Easawi, 2007). And that the pertubations induced by the water deficit are reversible at the cellular level in rice cultivars.

However these findings allow no discrimination between the cultivars used in this work since both cultivars recovered completely after the stress relief.

Cellular adaptation to such stress might be improved towards of intra cellular volume and cell turgor. It have been widly reported that cells achieve their osmotic adjustment by the accumulation of some kind of compatible solutes such as praline, betaine and polylos to protect membranes and proteins (Delauney and Verma, 1993).

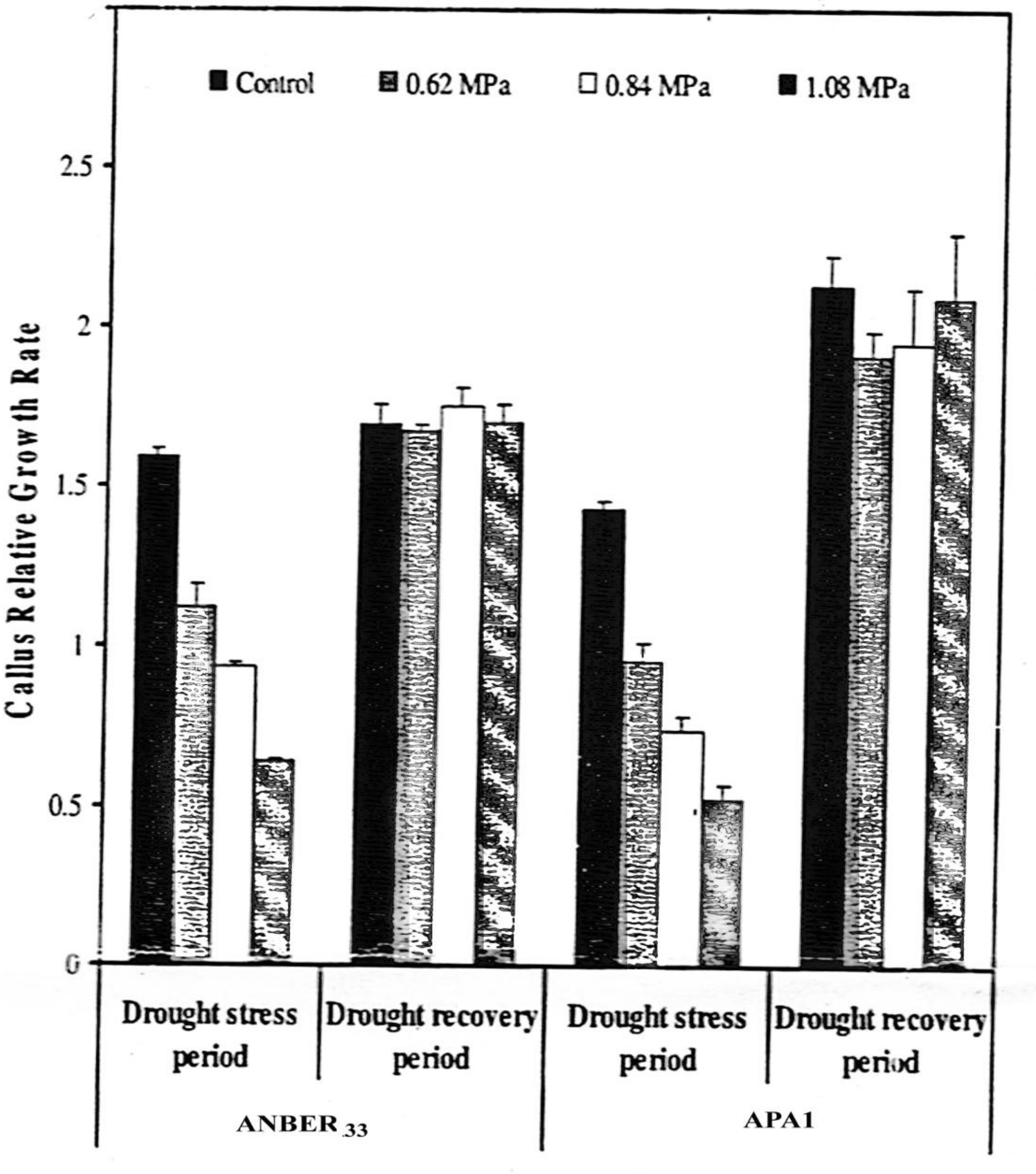

Figure 1. Callus relative growth of rice(Oryza SativaL.)Anber33 and APA1 calli after exposure to and recovery from mannitol-induced drought stress. Each value is the mean of six replicates and vertical bars represent ± standard error.

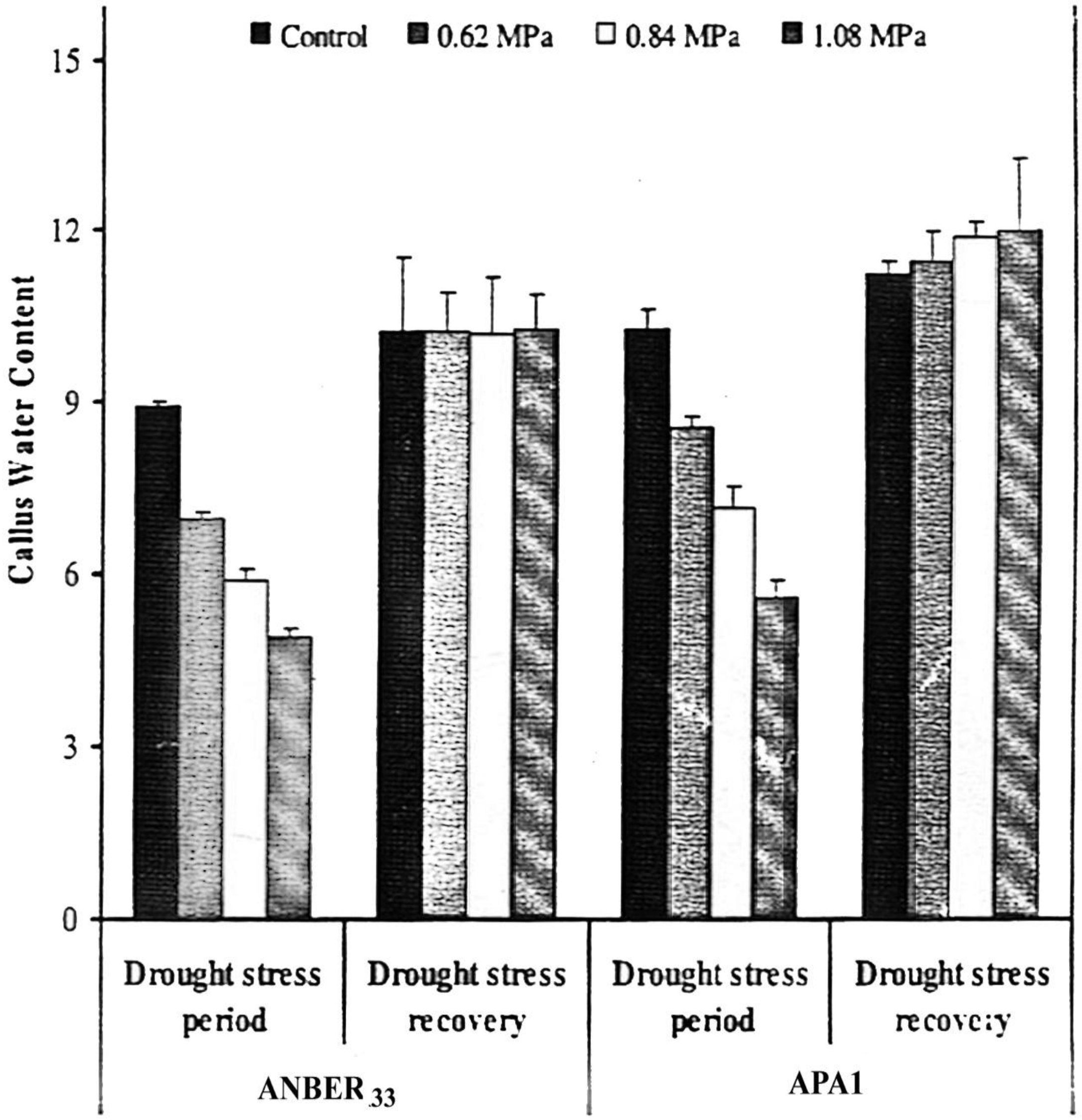

Figure 2. Changes in the water content of rice(Oryza SativaL.)Anber33 and APA1 calli after exposure to and recovery from mannitol-induced drought stress. Each value is the mean of six replicates and vertical bars represent ± standard error.

Consequently, there occurs an osmotic re-inflow of water into the cells.
Compatible solutes are over produced under osmotic stress aiming to facilitated osmotic adjustments (Hasegawa et al., 2000; Hummadi, 1994).

These compounds accumulated in high amount mainly in cytoplasm of stressed cells with out interfering with macromolecules and behaved as osmoprotectants (Yancey, 1994). Its has been shown that proline also have akey role in stabilizating cellular proteins and memberanes in presence of high concentration of osmoticum (Rudolph et al., 1986 ; Yancy, 1994 ; Torrecillos et al., 1995)

In our results, a highly significant difference was recorded in proline concentration ($F= 912.94$, $P<0.001$). thus, proline accumulation ratein callus culture increased drastically and proportionally to the increasing manuitol concentration (Figure3). Anber 33 calli accumulated less proline than IPA1 ones. At osmotic pressure of 1.08 Mpa, proline concentration increased by 10 Fold in Anber 33 calli and by 13 Fold in IPA! Calli. Although, these statements suggested that the proline is not directly involved in the drought-resistance, from aquantitative point of view rice cultivars at the least in the cellular level and corroborated with those reported in rice (Lutts et al.,1996) and wheat (Lutts et al., 2004; Al-Esawi,2007).

Calli growth recovery was accompanied with ruductian in proline concentration (Figure 3). The proline concentration decreased in both cultivars after the stress relief and returned to control levels. These findings suggested that organic solutes accumulated during the stress period are likely to be utilized for growth after stress relief. The praline has been suggested to play akey role after the stress relief. Thus, (Al-Esawi, 2007), (Ahmed and Wynjones, 1979) reported that the decreased in praline concentration during the recovery period was related to tissue rehydration. As well, it has been suggested serve as an organic nitrogen reserve ready to be used after stress relief to sustainth amino acid and protein synthesis (Trotel et al., 1996 ; Sairam and Tygui, 2004).

K^+ is major eation in cell organization and was reported to be a major contributor to osmotic adjustment under stress condition in several species (Bajji et al., 2001). Under mannitol induced osmotic stress K^+ concentration declined significantly ($F=282.49$, $P<0.001$) and it reached about 13 and 48% of the control Anber33 and IPA1 calli, respectively at 1.08Mpa (Figure 4). Thus, (Anber33) Accumulate more K^+ than (IPA1).

Similar results were reported earlier mannitol treated wheat callus (Al-Easawi, 2007; traivedi et al., 1991). And in mannitol-treated ricecallus (Lutts et al., 1996) While just slight decrease in K^+ content was observed in wheat callus (Bajji et al., 2000). The Ca^{2+} concentration

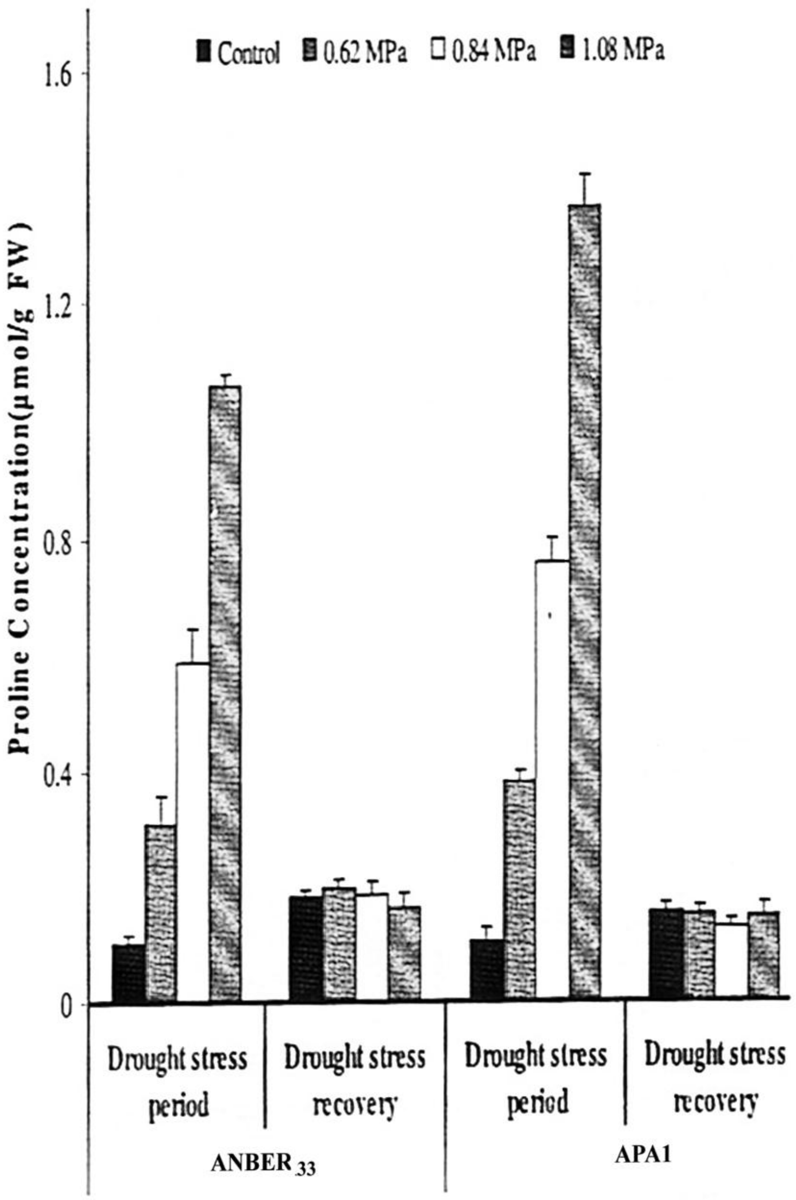

Figure 3. Changes in proline concentration of rice(Oryza SativaL.) Anber33 and APA1 calli after exposure to and recovery from mannitol-induced drought stress. Each value is the mean of six replicates and vertical bars represent ± standard error.

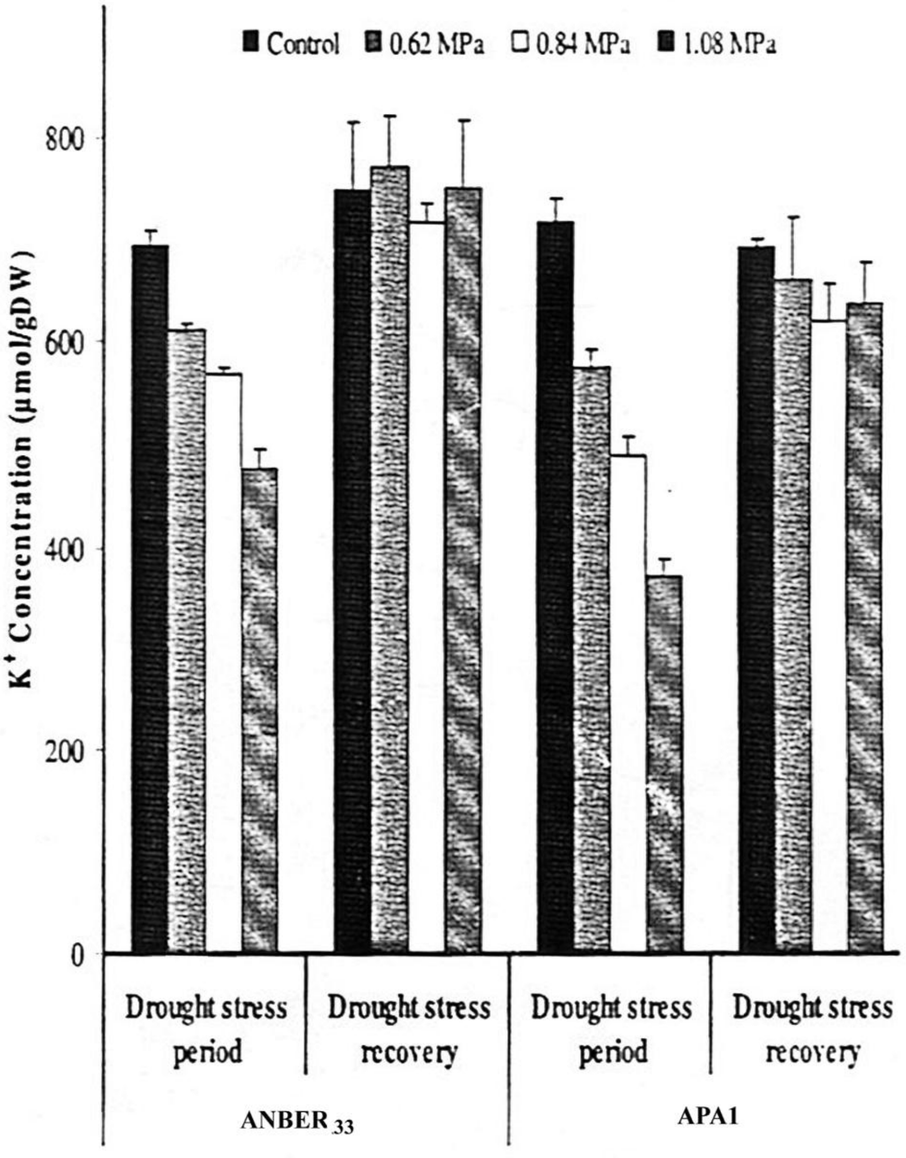

Figure 4. Changes in K^+ concentration of rice(Oryza SativaL.)Anber33 and APA1 calli after exposure to and recovery from mannitol-induced drought stress. Each value is the mean of six replicates and vertical bars represent ± standard error.

decreased significantly in the presence mannitol-supplemented of medium ($F=238.03$, $P<0.001$) and it reached to about 32%, 50%of the
control in (Anber 33 and IPA1), respectively. At cultivars, (Anber33) calli seemed to retain more Ca^{2+} than (IPA1) ones. The decrease of Ca^{+} content under osmotic stress has been previously reported in other species (Lutts et al., 1996). Besides, the role of Ca^{2+} in ensuring membrane integrity and the ion regulation within plant cells is well known (Hirschi, 2004). While, Na^{2+} and Mg^{2+} concentration were not significantly affected by osmotic stress whatever the mannitol concentration (Table 1).

Similar statements have been reported previously in some other species (Bajj et sl., 2000; Al-Easwai, 2007). While an important increase of Na^{+} concentration chilli pepper cell cultures exposed to PEG-induced stress was reported (Santos and Ochoa, 1994). After stress relief, Ca^{2+} and K^{+} concentration returned to the control levels at all the experimented mannitol doses in both cultivars (figures 4 and 5).

Comparative data on physiological changes in rice cultivars under drought stress and it is was recorded. Its appears that drought stress-induced changes are reversible, at the least at the cellular level, in rice cultivars since most of the investigated parameter returened to control levels in both cultivars irre spective of stress intensity according to the statical analysis at the end of recovery period. As well, our finding suggested the presence of cellular machinary that controls the recovery from the osmotic in duced stress in rice cultivars. (Tal,1983).

Table (1). Na^{+} and Mg^{2+} concentration in rice (oryza sativa L.) Anber33 and IPA1 calli exposure to mannitol-induced drought stress.

Mannito-induced drouht stress	Na^{+} concentration (mmol/9 dry weight)		Mg^{+} concentration (mmol/g dry weight)	
	Anber33	IPA1	Anber33	IPA1
Control	66.67 ± 8.17	156 ± 6.64	236.99 ± 5.09	285 ± 10.15
0.62 Mpa	72.5 ± 9.58	155 ± 12.91	239.39 ± 4.26	278.74 ± 17.44
0.84 Mpa	80 ± 16.32	148.75 ± 6.29	241.53 ± 5.68	282.1 ± 8.14
1.08 Mpa	70 ± 14.4	150 ± 10.80	238.91 ± 4.88	280.09 ± 12.95

Each value is the mean of six replicates ± standard error.

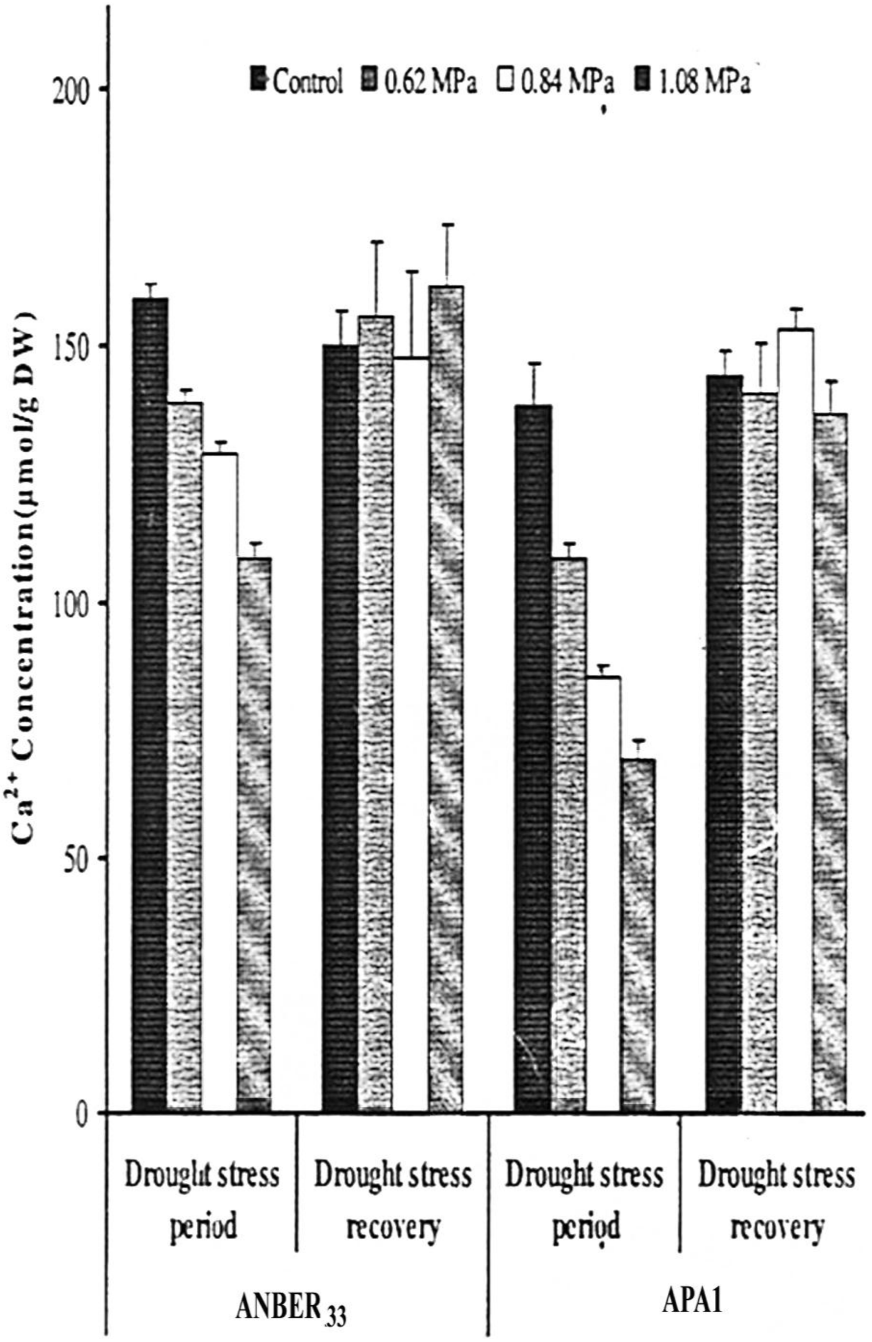

Figure 5. Changes in Ca^{2+} concentration of rice(Oryza SativaL.) **Anber33** and APA1 calli after exposure to and recovery from mannitol-induced drought stress. Each value is the mean of six replicates and vertical bars represent ± standard error

References

- Ahmad, N.R and Wyn, R.G (1979). Glycinbetaine, proline and inorganic ion barly seedlings following transient stress. Plant. Sci. lett. 15: 231-237.
- Al-Easawi, A.Y.H.(2007). Effected of salt stress on growth and regeneration of Triticum astivum L. in vitro. Babylon. Wniv. J. 14(3).
- Bajji, M.H., Lutts, S.R. and Kint, J.M. (2000). Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus culture issued from durum wheat (Triticum durum Desf.) Cultivars differing in drought resistance. J. plant. Physiol. 156: 75-83.
- Bates, L.S., Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. Plant and soil. 39:205-207.
- Bhaskaram, S.M., Smith, R.H., and Newton, R.J. (1985). Physiological changes in cultured sorghum cells in response to induced water stress. Plant. Physiol 79: 266-269.
- Boyer, J.S. (1982). Plant productivity and environment. Science. 218: 448-448.
- Delauny, A.J. and Verma, D.P. (1993). Proline biosynthesis and osmoregulation in plant plant J. 4: 215-223.
- Greenway, H.L. and Munns, R. (1980). Mechanisms of salts tolerance in nonhylophyter-Annu. Rev. plant physiol. 31: 146-190.
- Gandonou, A.J., Errabi, T., Aribini, J., and Chibi, F. (2005). Effect of genotype on callus induction and plant regeneration from leaf explants of sugar cane. Afr. J. Biotechnol. 4(11): 1250-1255.
- Hasegawa, P., Bressan, R.A., Zhu, J.K. and Bohnert, H.J. (2000). Plant cellular and molecular responses to high salinity. Annu. Rev. plant mol. Biol. 51: 463-499.
- Hirschi, D. (2005). The calcium conundrum. Both versatile nutrient nutrient and specific signal. Plant physiol. 136(1): 2438-2442.
- Kameli, A and Losel, D.M. (1996). Growth and sugar accumulation indurun wheat plants under water stress. New phytol. 132: 57-62.
- Kishor, P.B.K., Hong, Z., Miao, G.H., and Verma , D.A.S. (1995). Overexpression of phyrroline-5- carboxylate synthetase increase proline production and confers osmotolerance in transgenic plants. Plant. Physiol. 108(4): 1387-1394.
- Lutts, S. Kinet, J.M. and Bouharmont, J. (1996). Effect of various salts and of mannitol on ion and proline accumulation in relation to osmotic adjustment in rice callus cultures. J. plant. Physiol. 149: 186-195.
- Lutts, S. Alman Souri, M. and Kinet, J.M. (2004). Salinity and water stress have contrasting effect in the relationship between growth and cell viability during and after stress Exposure in durum wheat callus. Plant. Sci. 167: 9-18.
- Mohamed, M.A.H., Harris, P.J.C. and Helderson, J. (2000). In retro selection and characterization of adrought tolerant clone of tagetes minuta. Plant. Sci. 159: 213-222.
- Murashige, T. and Skooge, F. (1962). A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15: 473-497.
- Requin, R., and Lechasseur, P. (1979). Observations sur une methods de dosage de la proline libre dans les extraits de plant. Can. J. Bot. 57: 1851-1854.
- Perez, F., and Larher, F. (1995). Sucrose and proline accumulation and suger efflux in tomato leaf discs affected by NaCl and polyethylene glycol 6000 iso-osmotics stresses. Plant. Sci. 107: 9-15.
- Rhodes, D. and Samara, Y. (1994). Genetics control of dsmoregulation in plants. In cellular and molecular physiology of cell volume regulation. Strange K. Boca Raton: CRC Press.PP: 347-361.
- Rudolph, A.S., Crowe, J.H., and Crow, L.M. (1986). Effects three stabilizing agents: proline, betaine and trehalose on membrane phospholipids. Arch Biochem Biophys. 245: 134-143.
- Sairam, R.K. and Tygai, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci. 86: 407-421.
- Santos, M.S., and Ochoa, N. (1994). PEG tolerant cell colnes of chilli pepper (capsicumannum): Growth, osmotic potentials and solute accumulation. Plant cell Tiss. Org. cult. 37: 1-8.

- SAS Institute Inc (1992). SAS/STAT, J. users Gude. Release 6.03 edn. SAS Institute Inc. Cary, N.C., 1028.
- Tal, M. (1983). Selection for stress tolerance. In Evans, D.A., Sharp, W.P., Ammirato,P.V., Yamada, Y. (Eds): Hand book of plant cell culture (vol. 1). Techniques for propagation and breeding macmillan publishing co., New York. PP: 461-488.
- Torrecillas, A., Guillaume, C. Alarcon, J.J., and Ruiz, M.C. (1995). Water relations of two tomato species under water stress and recovery. Plant. Sci. 105: 169-176.
- Trivedi, S., Galiba, G., Sankhla, N., and Erdei, L. (1991). Responses to osmotic and NaCl stress of wheat varieties differing in drought and salt tolerance in callus cultures. Plant. Sci. 73: 227-232.
- Trotel, P., Bouchereau, A., and Larher, F. (1996). The fate of osmoaccumulated prolline in leaf discs of rape (Brassica napusl.) incubated in amedium of Low osmolarity plant. Sci. 118: 31-45.
- Yancey, P.H. (1994). Compatible and counteracting solutes. In cellular and mol. Pysiol. Of cell volume. Edited by strange K. Boca raton: C.R.C. Press; pp: 81-109.