

## Pre-exposure to gamma rays alleviates the harmful effect of drought on the embryo-derived rice calli

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### Abstract

The grains of *Oryza sativa* L. Sakha 101 cultivar (salt tolerant) were irradiated with 0.0, 5, 10 and 15 KR of gamma rays and their mature embryo-derived calli were exposed to different levels of drought-stress, in order to reveal the bases of physiological behavior of *in-vitro* cultured rice under gamma-irradiation or drought-stress. The effect of pre-treatment with gamma-irradiation on the response of rice to drought was also evaluated. The results showed that treatment with PEG highly significantly reduced the fresh and dry weight of the most investigated rice calli. However, the magnitude of loss was much less in the irradiated calli than the non-irradiated ones. For each increase in PEG-stress level there was a concomitant increase in total soluble sugars content of both the non-irradiated and irradiated samples. However, most of the irradiated calli exhibited higher tendency to accumulate soluble sugars than the non-irradiated ones in response to higher PEG concentrations. Though diminishing the content of most mineral elements by PEG, the irradiated cells could keep more of their phosphorus, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> than the non-irradiated ones. Polyamines (PAs) accumulated in rice calli under drought or radiation-stress. Accumulation of Put and Spd was higher than Spm in response to radiation-stress, while that of Spd and Spm was higher than Put in response to drought. While drought stress caused the disappearance of the 78 and 45 kDa polypeptides, radiation increased their expression. The 93 and 30 kDa polypeptides were expressed only under drought stress either with or without irradiation. The 69 and 60 kDa polypeptides were intensified in response to both radiation and PEG. It was concluded that the pre-exposure to gamma-irradiation had alleviated the harmful effect of drought on rice calli.

**Keywords:** *Oryza sativa* L.; gamma-irradiation; *in vitro* culture; sugars; minerals; polyamines; SDS-PAGE; PEG

**Abbreviations:** BA\_6 benzylaminopurine; 2,4-D\_2, 4-dichlorophenoxyacetic acid; IAA\_indole-3-acetic acid; KR\_ kilo rad; MS medium\_Murashige and Skoog medium; PAs\_polyamines; Put\_putrescine; Spd\_spermidine; Spm\_spermine

### Introduction

One possible way to ensure future food needs of the increasing world populations should involve a better use of water by the development of crop varieties which needs less amount of water and more tolerance of crops to drought (Shao et al., 2006). Therefore, spotting more light on the physiological mechanisms underlying drought stress is important to develop and introduce genetic or environmental enhancement to stress tolerance. Many researchers have used the *in vitro* culture of cells on media supplemented with

PEG to study the mechanism of drought tolerance and to utilize the somaclonal variation, as a source of variability, to improve the drought tolerance. PEG 8000 has been used to study the development of drought-tolerant plants and/or the morphological and physiological responses of plants under osmotic stress (van der Weele et al., 2000; Al-Khayri and Al-Bahrany, 2004). Because of its high molecular weight, PEG cannot cross membranes and cannot get into the cell to change its osmotic potential. It

stimulates water deficit conditions in cultured cells in a similar manner to that observed in the cells of intact plants subjected to true drought conditions (Attree et al., 1991).

Soluble sugars as one of the osmolytes commonly accumulate in plants growing under stressful conditions like salinity and water stress. These osmolytes reduce the internal osmotic potential and apparently prevent macromolecules from denaturation by supporting them to retain their natural configuration (Dubey and Pessarakli, 1995). Studying the response of mineral nutrients to drought stress is also important. It was suggested that membranes are the primary sites of desiccation injury to cells and organelles. Loss of membrane integrity was suggested by the increase of electrolyte leakage in black spruce under drought (Fan and Blake, 1994).

Polyamines (PAs) are polybasic aliphatic amines that are ubiquitous in prokaryotic and eukaryotic organisms. It has been well documented that PAs are closely associated with resistance of plants to water stress (Liu et al., 2006; Yang et al., 2007). The alteration of protein synthesis or degradation is one of the fundamental metabolic processes that may influence drought tolerance (Ouvrard et al., 1996). The overexpression of some proteins was found to be correlated with the drought tolerance, since it may contribute to the processes of detoxification, and osmotic and ionic homeostasis (Grover et al., 2001).

This work aimed to study the response of rice calli to radiation stress, drought stress or a combination of both in order to spot more light on the mechanisms underlying abiotic stresses. The work aimed also to evaluate the effect of gamma-irradiation on increasing tolerance of rice to drought stress.

## Materials and methods

Mature rice grains (Sakha 101, tolerant cultivar) were exposed to 5, 10 & 15 KR using Gamma Cell 220, model G.C.220 type B., Atomic energy of Canada Cobalt 60 at dose rate 19.8 rad/second provided by the Radiation Technology Centre, Nasr City, Cairo, Egypt. Two days after irradiation, the mature grains of irradiated and non-irradiated rice were dehusked, surface sterilized using 35% (v/v) of commercial bleach (active chlorine 52.5 g/l) for 30 minutes and rinsed several times with sterilized distilled water. After soaking the grains for 12 hours, the embryos were excised aseptically and cultured under sterile conditions on MS medium (Murashige and Skoog, 1962) supplemented with 8 g/l agar, 2 mg/l of 2, 4-dichlorophenoxyacetic acid (2,4-D) and 3% (w/v) sucrose. The medium pH was adjusted to 5.8 before the addition of agar and then autoclaved at 121 °C and 15 psi for 20 minutes. Cultured embryos were

maintained in dark at  $25 \pm 2$  °C and 70% humidity for 4 weeks to induce calli. Four weeks-old calli were subdivided into pieces each of 200 mg and inoculated on the basal MS media supplemented with 2 mg/l 2,4-D, 0.5 mg/l 6 benzylaminopurine (BA), 3% sucrose, different levels of osmotic-stress (0.0, 5, 10 and 15% PEG) and 2.5 g/l phyta-gel. Cultures were kept in dark at  $25 \pm 2$  °C and 70% humidity, and sub-cultured every 6 weeks on the same corresponding media in order to get selected cell lines. After 6 months the fresh and dry weight/callus of all investigated calli was determined as percentage of control (5 replicates). The dried materials were powdered and kept for the estimation of soluble sugars and mineral contents. The rest of the calli were kept for the analysis of polyamines and protein electrophoresis.

## Biochemical Analyses

### Estimation of total soluble sugars

Total soluble sugars were extracted in 80% hot ethanol and estimated by the anthrone reagent method (Yemm and Willis, 1954). The content of the soluble sugars was calculated as mg glucose g<sup>-1</sup> dry weight, using glucose standard curve.

### Estimation of Mineral Contents

#### Extraction method

A known weight of dried powder tissue was wet-acid digested in a mixture of nitric and perchloric acids for complete oxidation. A known aliquot of the digested extract was neutralized and kept for estimation of total nitrogen and phosphorus. The rest was used for estimation of sodium, potassium, calcium and magnesium.

### Estimation of total nitrogen

Total nitrogen was estimated at the neutral wet digested extract as ammonia-N using Berthelot reaction (Chaney and Marbach, 1962). The optical density of the developed colour was determined spectrophotometrically at 630 nm. Nitrogen content was estimated as mg nitrogen g<sup>-1</sup> dry weight, using a standard curve of ammonium chloride.

### Estimation of total phosphorus

The modified Fiske-Subbarow method described by Clark and Switzer (1977) was used to estimate the phosphorus content in the neutral wet digested extract. The ammonium molybdate-sulphuric acid reagent and the reducing metal reagent were used in

**Table 1.** Effect of gamma-irradiation (0.0, 5, 10 and 15 KR) and PEG (0.0, 5, 10 and 15 %) on total N, P, K, Ca and Mg contents of sakha rice calli. Values listed are expressed as (mg element g<sup>-1</sup> dry weight).

Element	Gamma Dose (KR)	% PEG									
		0		5		10		15			
N	0	17.96	± 0.37	20.96	± 1.54	12.64	± 0.33	9.4	± 0.07		
	5	24.01	± 1.03	18.50	± 0.08	18.12	± 0.71	18.34	± 0.84		
	10	20.71	± 0.44	20.37	± 1.43	19.10	± 0.72	22.02	± 0.80		
	15	15.00	± 0.00	20.58	± 0.13	20.15	± 0.19	4.94	± 0.11		
P	0	3.58	± 0.11	2.90	± 0.05	1.14	± 0.03	0.50	± 0.02		
	5	2.81	± 0.05	2.03	± 0.03	2.03	± 0.02	2.23	± 0.16		
	10	3.75	± 0.13	2.27	± 0.05	2.95	± 0.09	2.35	± 0.03		
	15	3.20	± 0.00	2.52	± 0.05	2.45	± 0.02	0.45	± 0.05		
K	0	31.11	± 1.00	17.64	± 0.80	10.73	± 0.30	2.32	± 0.40		
	5	27.97	± 0.40	15.70	± 0.50	15.40	± 1.50	15.39	± 2.00		
	10	27.49	± 0.00	18.06	± 0.30	18.69	± 1.20	15.80	± 0.80		
	15	25.00	± 0.40	21.19	± 0.20	17.21	± 0.30	6.29	± 1.40		
Ca	0	0.067	± 0.003	0.057	± 0.004	0.058	± 0.000	0.053	± 0.000		
	5	0.075	± 0.003	0.058	± 0.004	0.057	± 0.000	0.062	± 0.000		
	10	0.070	± 0.000	0.063	± 0.007	0.078	± 0.002	0.068	± 0.000		
	15	0.090	± 0.006	0.063	± 0.002	0.068	± 0.003	0.052	± 0.000		
Mg	0	0.873	± 0.050	0.628	± 0.040	0.562	± 0.000	0.226	± 0.005		
	5	1.645	± 0.004	0.590	± 0.020	0.510	± 0.050	0.597	± 0.000		
	10	0.978	± 0.000	0.617	± 0.010	0.432	± 0.020	0.624	± 0.010		
	15	1.200	± 0.030	1.556	± 0.100	0.794	± 0.060	0.214	± 0.040		

this method. The color intensity was recorded at 660 nm against water reagent blank. The phosphorus content was obtained from a potassium dihydrogen phosphate standard curve and calculated as mg phosphorus g<sup>-1</sup> dry weight.

#### **Estimation of potassium, calcium and magnesium**

Potassium, calcium and magnesium determination was carried out using the atomic absorption spectrophotometer (GBC 932 AA).

#### **Estimation of Free Polyamines**

Putrescine, spermidine and spermine were extracted and determined according to Mietz and Karmas (1977) and Majjala and Eerola (1993) with some modifications. For extraction of polyamines, about one gram fresh tissue sample was homogenized in 5% (w/v) TCA and then filtered. Ten milliliters of the

extract were transferred into a culture tube with 4 g NaCl and 1ml of 50% (w/v) NaOH, shaken and extracted three times by 5 ml n-butanol/chloroform (1: 1, v/v). Centrifugation was carried out for 5 min, at 3000 rpm. The upper layer was extracted three times with n-heptane and 0.2N HCl. The HCl layers were collected and then evaporated just to dryness. The residues were derivatized with dansyl-chloride solution (5 mg ml<sup>-1</sup> acetone) and extracted with diethylether.

After dansylation, the dansylamines were determined by HPTLC densitometer by separating PAs on thin layer chromatography (TLC) plates with chloroform: benzene: triethylamine (6: 4.5: 1). A Camag Linomat IV (Camag, Muttenz, Switzerland) was used for spotting the the plates and a Camag TLC Scanner VI (Camag, Muttenz, Switzerland) for scanning the plates. Data were acquired and processed using a CATS software (V4. 04). The final data were calculated as µg polyamines g<sup>-1</sup>fresh weight.

### ***Extraction and Estimation of Soluble Proteins***

Fresh tissue samples (1 g) were placed into liquid nitrogen and then homogenized under ice cold-conditions in 4 ml 50 mM potassium phosphate buffer, pH 7.0, with the addition of 1 mM EDTA and 1% (w/v) insoluble polyvinylpoly-pyrrolidone. The homogenate was centrifuged at 12000 rpm, at 4 °C for 30 min. The supernatant was used for soluble protein assay and protein electrophoresis. Protein content was determined spectrophotometrically at 595 nm using Coomassie dye reagent (Bradford, 1976). Bovine serum albumin was used as a standard at concentrations 20-140 µg of protein.

### ***SDS-PAGE Protein Electrophoresis***

A volume of the soluble protein extract (containing 100 µg protein) was added to an equal volume of 10% (w/v) TCA, mixed well and left on ice for 20 min. The mixture was centrifuged for 15 min at 10,000 rpm. The pellets were washed with 75% (v/v) ethanol, left to dry and then re-suspended in 50 µl of 0.1 M Tris-HCl pH 7.5. A volume of protein sample (5 µl, containing 10 µg protein) was added to an equal volume of the sample buffer (20% (v/v) glycerol, 0.125 M Tris-HCl pH 6.8, 4% (w/v) SDS, 10% (v/v) 2-mercaptoethanol, and 0.02% (w/v) bromophenol blue) and mixed well. Samples should be incubated in sample buffer for up to 3 minutes at 90 °C just prior to loading. SDS-PAGE separation was carried out according to the method of Laemmli (1970) in 12% polyacrylamide gel, using the apparatus Bio-Rad mini protein gel electrophoresis. Gels were stained with Coomassie Brilliant Blue. The de-stained gel was scanned and analyzed using a Densitometer Gel Proanalyzer 3.1 for version 95 N/T, Media Cybernetics (1993-1997).

### ***Statistical Analyses***

Statistical analysis was performed using the SPSS statistical package (version 17.0). Morphological data were tested at significance levels of  $P < 0.05$  by two-way ANOVA to investigate the effect of different levels of salinity, different doses of gamma-radiation and the interaction of both on the growth of Sakha 101 rice calli. Mean values were compared with a least significant difference test (LSD) at 1 and 5% significance. Values reported for fresh and dry weights are means of five independent replications. Data of the biochemical analyses were calculated as means of three replicates  $\pm$  standard error (SE).

## **Results and discussion**

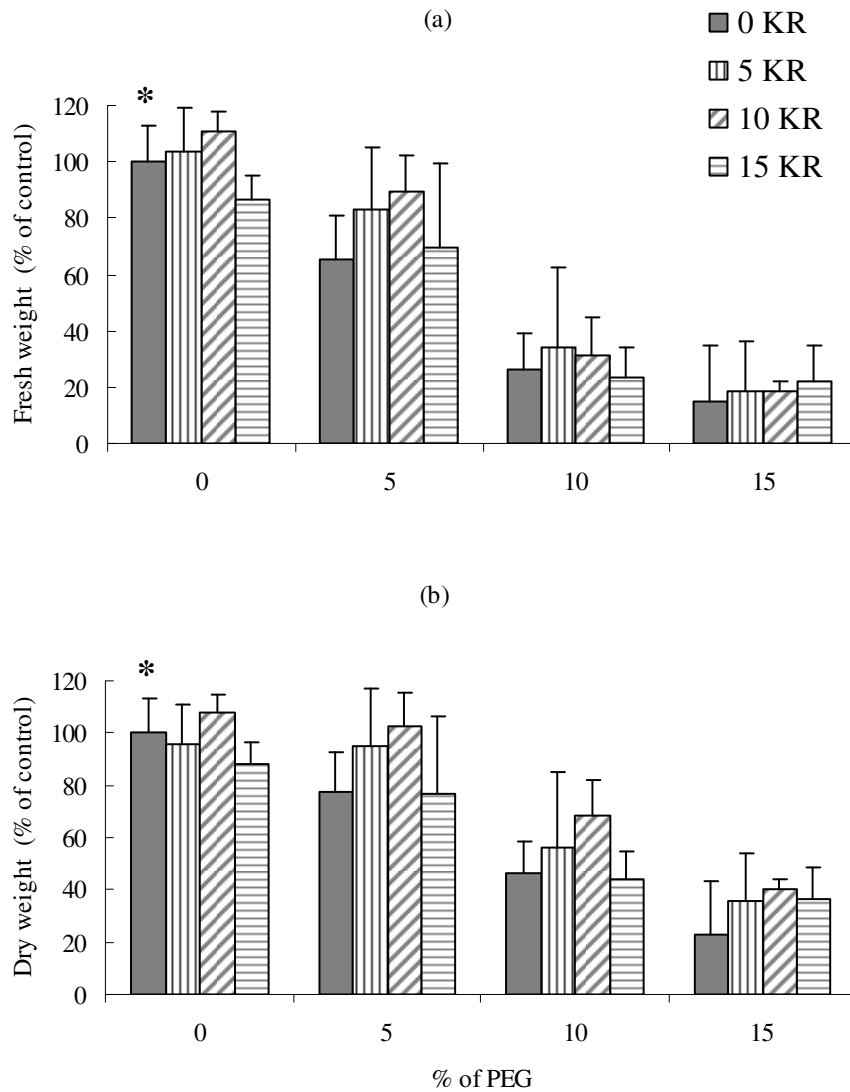
In this work the growth parameters and the physiological behavior of embryo-cultured calli were studied in response to application of osmotic stress (5, 10 & 15% PEG), gamma-irradiation (5, 10 & 15 KR) and a combination of both, compared with the untreated control (0.0% PEG + 0.0 KR).

### ***Growth parameters***

The application of gamma-rays (5, 10 and 15 KR) alone non-significantly changed the fresh weight and dry weight of Sakha 101 calli, respective to control (Fig 1). Osmotic stress due to PEG application highly significantly decreased the fresh weight of the non-irradiated calli as well as the irradiated ones in response to 10 & 15% PEG, as compared with the control (Fig 1a). It is apparent also from data that 5% PEG concentration highly significantly decreased the fresh weight of the non-irradiated calli by 34.5% below the control, whereas significantly decreased those irradiated with 5, 10 and 15 KR by 17.3, 10.5 & 30.3% respectively, below the control.

Treating with PEG caused nonsignificant inhibition at 5% and high significant inhibition at 10 and 15% in the dry weight of the most irradiated and non-irradiated calli (Fig 1b). However, the magnitude of inhibition was much less in case of the irradiated calli than in the non-irradiated ones. The loss in dry weight of the non-irradiated calli due to 5, 10 and 15% PEG treatment was 22.8, 53.7 & 76.8%, while it was 4.8, 43.9 & 63.9% in 5 KR-irradiated calli below the control respectively. The most pronounced effect was due to application of 10 KR dose in combination with PEG which increased the dry weight nonsignificantly by 2.2% over the control in response to 5% PEG and reduced the loss in dry weight to 32 & 59.5% below control in response to 10 & 15% PEG respectively (Fig 1b). These data reveal the obvious role of gamma-irradiation in protecting the calli against the inhibitory effect of PEG-stress on their growth.

The reduction in the growth (fresh and dry weights) of rice calli, detected in our experiment as a result of treatment with PEG, is consistent with those found in sunflower and maize (Navari-Izzo et al., 1990). In the current study, the observed decline in fresh weight and dry weight (Fig 1) in PEG environment may be as a sequel to consuming their C and N in defense, osmoregulation and repair.



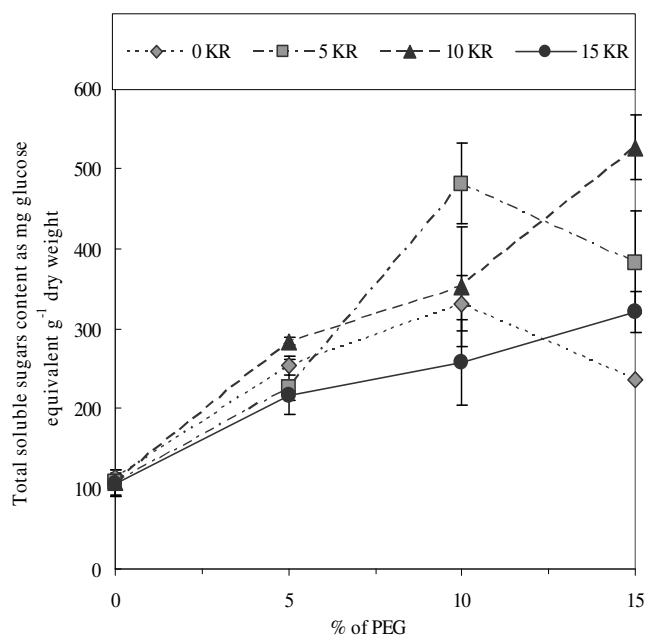
**Fig 1.** Effect of gamma-irradiation (0.0, 5, 10 and 15 KR) and PEG (0.0, 5, 10 and 15 %) on (a) fresh weight and (b) dry weight of sakha rice calli. Values listed are expressed as % of control  $\pm$  SE

	(a)		(b)	
	LSD at 5%	LSD at 1%	LSD at 5%	LSD at 1%
Radiation	43.00	56.51	42.81	56.26
PEG	17.51	23.01	23.58	30.99
Radiation $\times$ PEG	16.02	21.05	20.14	26.47

### Total Soluble Sugars Content

It is clear from Fig (2) that the application of gamma-irradiation did not change the content of total soluble sugars of the calli cultured on free PEG- media. The increase in total soluble sugars content was directly proportional to the increase of PEG-stress in both the non-irradiated and the irradiated samples (Fig 2).

Most of the irradiated calli exhibited higher tendency to accumulate soluble sugars than the non-irradiated ones in response to higher PEG concentrations (10 & 15% PEG). There is no doubt that total soluble sugars exert a positive role in the alleviation of the imposed stress via osmotic adjustment in plants (Kameli and Losel, 1996; Kerepesi and Galiba, 2000). Some of the soluble sugars accumulate in stressed cells to



**Fig 2.** Effect of gamma radiation and PEG on total soluble sugars content of rice calli. Each value is a mean of 3 replicates. The bars indicate  $\pm$  SE

maintain membrane phospholipids in the liquid-crystalline phase and to prevent structural changes in soluble proteins and others play a key role in stress-induced metabolic processes (Kerepesi and Galiba, 2000). Substantial osmotic adjustment was observed in adapted soybean cell suspension cultures exposed to water stress, due mainly to increased glucose, fructose and sucrose (Dubey and Pessaraki, 1995).

Although the content of total soluble sugars nonsignificantly changed by the application of gamma-irradiation alone, it increased in much pronounced levels in response to a combined stress of irradiation and drought than in response to drought stress alone. This behavior confirms that increasing osmolyte/osmoprotectant contents may come as another defensive mechanism comes via the activation of genes responsible for expression of the enzymes involved in the accumulation of these osmolytes which caused by the pre-exposing to gamma rays, leading to more protection for the up-regulating enzymes involved in the anabolism of these contents.

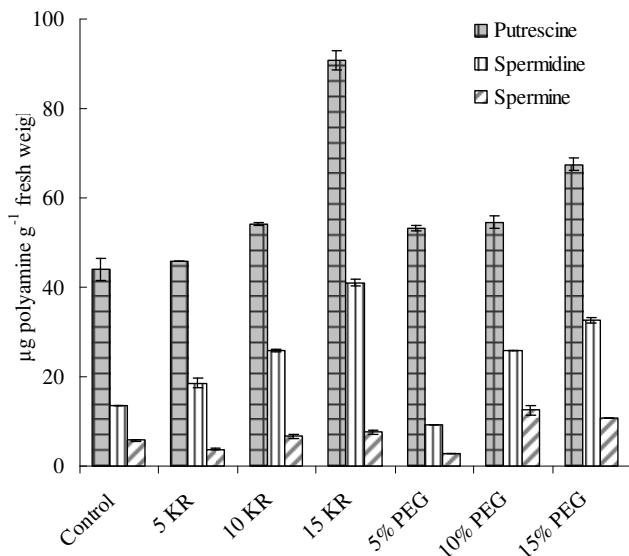
### Mineral Contents

The results of total nitrogen represented in Table (1) indicate that irradiation with 5 and 10 KR doses of gamma-rays increased markedly the total nitrogen content while the 15 KR dose decreased it as

compared with the control. At lower level of drought stress alone (5% PEG) the content of nitrogen increased by 16.7% over the control, while it decreased sharply by 29.6 and 47.6% below the control at excessive water stress (10 and 15% PEG respectively). The exposure of Sakha rice cells to variable doses of gamma-rays before culturing on PEG has slightly stimulated, in most cases, the accumulation of total nitrogen in these cells relative to the control. The increase in total nitrogen, especially at the irradiated cells, in response to PEG-induced stress may be due to the increase in protein synthesis or to increase in nitrate absorption and assimilation.

Concerning the changes in phosphorus content, Table (1) shows that all stresses (radiation alone, drought alone and radiation X drought stress) generally decreased phosphorus content in Sakha rice cells as compared with the control. The Table shows also that the application of gamma-irradiation reduced the inhibition of phosphorus accumulation in drought-stressed rice cells, especially at the high concentrations of PEG (10% and 15%). For example, at 10% PEG concentration, phosphorus content decreased by 43.5, 17.7 and 31.6% below the control in the 5, 10 and 15 KR-irradiated calli respectively, while it decreased by 68.3% in the non-irradiated ones. Drought-induced phosphorus deficiency appears to affect metabolism of the adenylated nucleotides, ADP and ATP (Bajji et al., 2000). Stonov and Petinov (1980) suggested that this could be a cause of the early reduction in growth that occurs before loss of turgor. On relief of water stress, levels of  $P_i$  in leaves of *Capsicum* and *Lolium* do not recover as rapidly as does growth (Turner, 1985). The trend of change in  $K^+$  content was more or less similar to that of phosphorus, under the same stresses (Table 1). The results of  $K^+$  content were also similar to those found by Turner (1985) and Thomas (1991), who reported that  $K^+$  has been decreased in cells of osmotic stressed wheat callus.

The contents of  $Ca^{2+}$  and  $Mg^{2+}$  increased markedly in the cells cultured on PEG-free media by increasing the dose of gamma-irradiation as compared with the control (Table 1). In contrast, the accumulation of  $Mg^{2+}$  was inhibited in the non-irradiated cells by increasing PEG concentration in the outer environment, while that of  $Ca^{2+}$  was slightly decreased. The irradiation with gamma-rays not only reduced the loss of  $Ca^{2+}$  caused by PEG-stress in some treatments but also increased the content of this mineral in others as compared with the control;  $Ca^{2+}$  content increased by 16.5 and 2.3% over the control in the 10 and 15 KR-irradiated cells respectively in response to 10% PEG concentration (Table 1). The interaction between drought and irradiation stress has



**Fig 3.** Effect of gamma radiation or PEG on polyamines content in rice calli. Each value is a mean of 3 replicates. The bars indicate  $\pm$  SE.

decreased, in most cases, the content of  $Mg^{2+}$  in rice cells as compared with the control.

The observed inhibition in the accumulation of phosphorus,  $K^+$  and  $Mg^{2+}$  contents in rice cells in response to PEG stress was similar to that reported by Lutts et al. (2004), who found that levels of  $K^+$ ,  $Mg^{2+}$  and phosphorus tended to slightly decrease in durum wheat calli with increasing PEG concentration in the medium. They suggested that all these solutes did not make any contribution to osmotic adjustment. The effect of gamma-rays application in reducing the loss of  $K^+$  and  $Ca^{2+}$  contents of rice cells in response to drought stress indicate that irradiation positively increased the involvement of these minerals in the osmotic adjustment inside the cells against the PEG-induced-low water potential in the outer environment.

### Free Polyamine Contents

The irradiation with gamma-rays has stimulated mostly the contents of Put, Spd and Spm in rice calli as compared with their respective controls (Fig 3). The accumulation of each of them increased as the dose of irradiation increased. The total polyamines increased by 107.9, 136.9 and 220.2% over control in response to 5, 10 and 15 KR, respectively. Among the investigated polyamines, Put and Spd were accumulated in higher levels than Spm in response to irradiation stress (Fig 3). The accumulation of Put has been widely reported in monocotyledonous and dicotyledonous plants but is most pronounced in

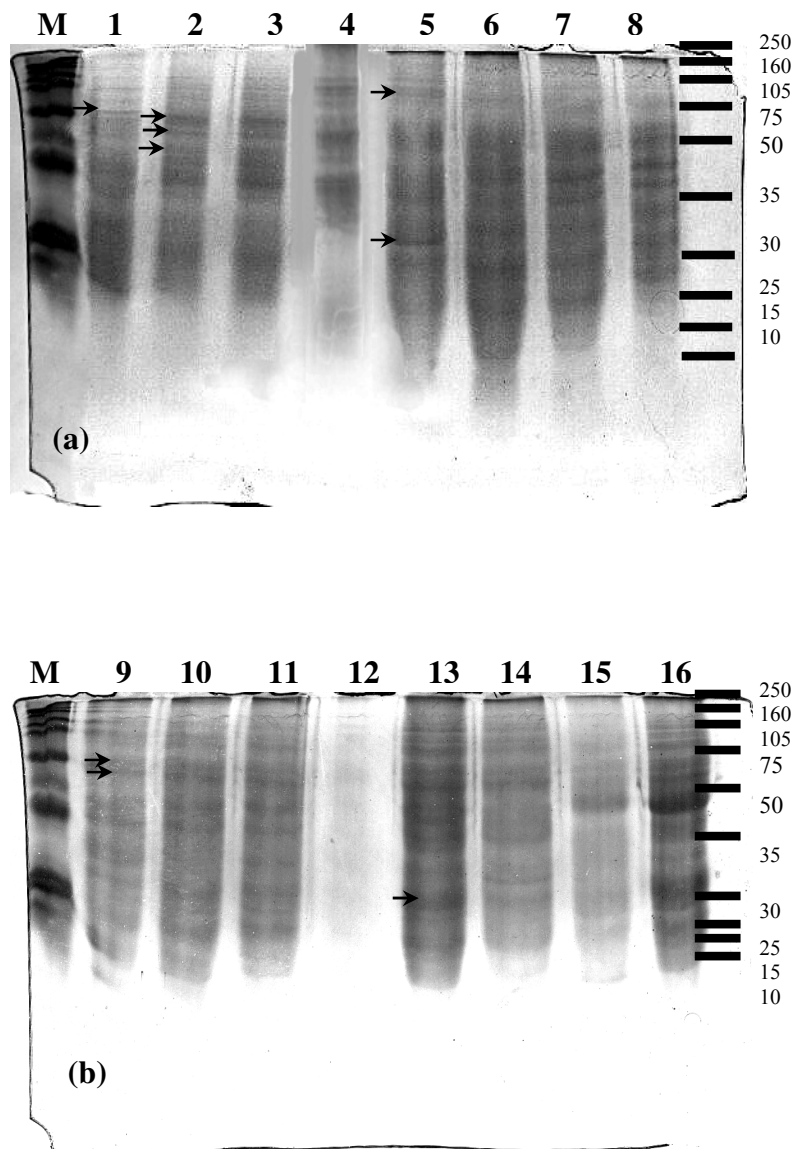
cereals; where the putrescine pool represents a major sink for carbon and nitrogen (Bajji et al., 2000). Put accumulation also may be as an oxidative stress response. UV light has been shown to stimulate Put accumulation in cucumber (Slocum and Weinstein, 1990).

It could be seen from the results that rice calli cultured on 5% PEG concentration exhibited an increase in Put by 21% over the control, while they exhibited a decrease in Spd as well as Spm by 32% and 51% below the respective controls (Fig 3). More increase in PEG concentration induced an increase in the content of free Polyamines (Put, Spd and Spm) of rice calli as compared with those of the controls. The results indicated that Spd and Spm (increased by 140 and 88% over control respectively) were more affected and accumulated in response to the high levels of drought stress (15% PEG) than Put (increased by 53% over control), indicating that Spd and Spm have a role in the acclimation of plant to water limitation (Liu et al., 2006; Yang et al., 2007).

The general trend of change indicated that each of Put, Spd or Spm and subsequently total free polyamines increased in response to drought as well as radiation stress. The response of Put and Spd was higher than Spm in case of radiation stress, while the response of Spd and Spm was higher than Put in case of drought stress. The trend also showed that (Spd+Spm)/put ratio was the highest at 10 KR dose in case of radiation stress (0.6) and at 10% PEG in case of drought stress (0.7), concluding the role of Spd and Spm in acclimation of plant to different types of abiotic stresses. Because of their polycationic nature at physiological pH, polyamines are able to interact with proteins, nucleic acids, membrane phospholipids and cell wall constituents, thereby activating or stabilizing these molecules (Duan et al., 2008). Moreover, it has been well documented that PAs are closely associated with resistance of plants to water stress (Bratton, 1994). Spd and Spm might interact with membranes by inhibiting transbilayer movement of phospholipids (Bratton, 1994).

### Protein Electrophoresis

Our SDS-PAGE protein analysis (Fig 4 a and b) showing that exposing rice cells to drought stress caused the disappearance of the 78 and 45 kDa polypeptides, while caused the induction and accumulation of the newly synthesized polypeptides (93 and 30 kDa). On the contrary, the treatment with gamma rays only had no role on the expression of 93 and 30 kDa polypeptides and increased the expression of 78 and 45 kDa ones. The 30 kDa protein may be similar to that reported by Tiburcio et al. (1993) who found that intensity of the 29 kDa polypeptide was



**Fig 4.** The SDS-PAGE profiles of soluble protein extracted from rice calli under radiation and/or drought stress. Arrows indicating major differences in bands of (a) 93, 78, 69, 60, 45 and 30 kDa and (b) 69, 60 and 30 kDa from top to bottom. (M) Protein marker. 1- 0.0 KR+0.0% PEG, 2- 5 KR+0.0% PEG, 3- 10 KR+0.0% PEG, 4- 15 KR+0.0% PEG 5- 0.0 KR+5% PEG, 6- 5 KR+5% PEG, 7- 10 KR+5% PEG, 8- 15 KR+5% PEG, 9- 0.0 KR+10% PEG, 10- 5 KR+10% PEG, 11- 10 KR+10% PEG, 12- 15 KR+10% PEG, 13- 0.0 KR+15% PEG, 14- 5 KR+15% PEG, 15- 10 KR+15% PEG, 16- 15 KR+15% PEG

enhanced in tobacco water stress-adapted cells, while the 93 kDa polypeptide may belong to HSP 90. Expression of HSP 90 in plants has been correlated with diverse functions such as flowering, pathogenesis, and possibly tolerance to abiotic stresses like high and low temperatures, salinity and drought (Iraki et al., 1989).

The 69 and 60 kDa polypeptides were intensified in response to both types of stresses (radiation and PEG

stresses). Since these two polypeptides were overexpressed by the preexposure to gamma rays, it was logic that their intensities were higher in the irradiated cells than in the non-irradiated ones in response to PEG. This behavior may indicate the effective role of gamma-irradiation in reinforcing the tolerance of rice cells against the drought stress.

The expression of many proteins is known to be regulated by biotic and abiotic stresses, suggesting



the occurrence of complex mechanisms that control genes expression in response to environmental stresses. In our study, irradiation and drought stresses had contrary effects concerning the 93, 78, 45 and 30 kDa polypeptides, indicating that those proteins may be associated with osmotic balancing and not with irradiation-induced oxidative stress. Both radiation and drought stresses stimulated the expression of 60 and 69 kDa polypeptides and the expression of these polypeptides was more in the irradiated rice cells than the non-irradiated ones in response to PEG, indicating that these proteins may be associated with the common mechanisms shared by both stresses. Immunological studies must be done to specify the role of the above proteins and to confirm that they are involved in the stress tolerance.

Conclusively, our study confirmed the effective role of gamma-irradiation in increasing the tolerance of rice cells against drought through increasing the efficiency of regulating the osmotic and ionic homeostasis. This may come also via mounting PAs which are able to moderate the activities of scavenging system enzymes and to influence oxidative stress intensity (Kubis, 2008).

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