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EFFECT OF GAMMA RAYS, ABSCISIC ACID AND PUTRESCINE ON PRODUCTION OF WHEAT PLANTS MORE TOLERANT TO SALINITY:

B- *IN VITRO* CALLUS INDUCTION, PLANT REGENERATION, AND GRAINS PRODUCTION UNDER SALINE CONDITIONS

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ABSTRACT

In vitro tissue culture experiment was conducted to evaluate the extent of improvement in salinity tolerance of the sensitive Giza 167 cultivar due to the previously applied treatments in the pot experiments during the first part (A) of the present investigation. The embryonic calli, plantlets and plants regenerated from the grains produced either by the salt-sensitive untreated and treated plants of Giza 167 cultivar or by the untreated plants of Sakha 8 one, in the second pot experiment season; 2000-2001 were used to be tested *in vitro* under the same salinity levels previously applied in the first part of this study. The obtained results from the *in vitro* experiments were in full accordance with those obtained in the first part, as regards the absolute superiority of the regenerated calli, plantlets, and plants from the mature grains of the previously treated plants (in the pot experiments) with the weekly spraying either with 10μM putrescine or 2 ppm ABA as well as the grains irradiation with 15 KR gamma rays in their growth, chemical composition (in both tissues and grains), and invertase activity as well as their grain yield (g)/plant and 1000 grains-weight which recorded the highest highly significant increments over the respective values of those produced from the grains of the salt-tolerant Sakha 8 control, also, up to 30% sea water level, nevertheless, without applying any treatments under such *in vitro* conditions. This finding strongly indicating the possibility of transmission of the physiological tolerance to salinity induced by those treatments previously applied in the pot experiments in the first part with nearly equal extent from the parent tolerant plants to their regenerations under *in vitro* conditions, thus enable them to be adapted to the all applied levels of salinity in the media and therefore, could complete their life cycle till harvest and grains production which were highly significantly exceeded that both controls also up to 30% sea water under *in vitro* conditions, as did under pot experiments conditions in the first part. Therefore, the obtained results strongly suggested that the tolerance to salinity of the salt-sensitive Giza 167 wheat cultivar potted plants and even their regenerated plants under *in vitro* conditions, can be improved to a considerable extent, thus could tolerate the irrigation with saline water up to 30% sea water and nevertheless, attained their optimal productivity which highly significantly exceed the productivity of the salt-tolerant Sakha 8 cultivar control under the same 30% salinity level in both cases, if these sensitive plants were weekly sprayed either with 10 μM putrescine or 2 ppm ABA or when their grains were irradiated with 15 KR gamma rays before sowing only in the pot experiments, with special referring to the putrescine treatment in this concern.

INTRODUCTION

Wheat [*Triticum aestivum* L.] is the most important cereal winter crop in Egypt. The amount needed for the local consumption is greater than the total productivity from this crop in Egypt. Thus, increasing its productivity and the cultivated areas are highly demanded. Increasing wheat productivity per

unit area can be achieved through the applying of some specific physiological treatments (such as gamma and laser rays irradiation as well as with fast neutrons, biofertilizer treatments, soil and foliar fertilization in addition to the exogenous application of the plant growth regulators) in addition to the expansion of wheat cultivation in the newly reclaimed areas. On the other hand, and due to the restricted resources of the fresh water from the River Nile, the use of saline water or even diluted sea water becomes the only source of irrigation water in such newly cultivated areas, but the sensitivity of some wheat cultivars to salinity will restricts or even prevents their cultivation in such reclaimed areas. Thus, it is mandatory to improve the salinity tolerance of such sensitive wheat cultivars and consequently enhancing their ability to tolerate salinity which, in turn, increasing the possibility of their successful cultivation in such newly reclaimed areas. Therefore, there is increasing attention to accommodate wheat cultivars to grow in salinities outside their natural range of tolerance and nevertheless obtain appropriate economic productivity. Moreover, great efforts are being directed towards the development of salt-tolerant crop genotypes through the use of plant breeding strategies involving the introgression of the genetic background from saline-tolerant wild species into cultivated plants. With recent developments in biotechnology, there is also the potential for obtaining salt-tolerant crop genotypes by the use of somatic cell selection or protoplast fusion or by gene transformation using recombinant DNA methodologies. (Hayashi *et al.*, 1997).

On the other hand, cell culture may offer many advantages for isolation of mutants in higher plants. Unlike the whole plant, a very large number of cells can be screened at one time for a described trait. Because the cell is grown in a uniform cultured environment, reproducible selection schemes can be employed. Efficient mutagensis of plant cell cultures is possible, as the cells can be uniformly treated with physiological mutagen. Mutant cell lines have been selected which have a direct application in both fields and basic researches in plant genetics (El-Shihy *et al.*, 1994a). Moreover, plant tissue and cell culture techniques are effective tools for producing salt tolerant cell lines, tissues and plants as well as the evaluation of the improving extent in the tolerance to salinity in the sensitive plant materials which offers several advantages during the physiological studies. In this concern, Yang *et al.* (1990) found that growth and Na^+/K^+ ratio in salinized calli were correlated with the previously determined whole plant responses. Thus, they concluded that callus growth and Na^+/K^+ ratio could therefore, be used as indicators of whole plant salt tolerance in sorghum. Similarly, El-Hennawy (1996) stated that the significant differences in calli growth rates of 11 wheat cultivars in response to salt stress. Moreover, Bhaskaran *et al.* (1986) suggested that sorghum seedlings from the salt-selected plant obtained adequate nutrients to allow higher shoot dry matter accumulation than that of the parent population.

Therefore, the aim of the present study was to evaluate the extent of tolerance improvement in the salt-sensitive Giza 167 wheat cultivar due to the previously applied treatments in the pot experiments in the first part (El-Shafey *et al.*, 2003a) of this investigation. Thus, the generated calli, plantlets

and plants from the embryos of the mature grains produced by the all treated plants as well as both sensitive and tolerant controls in the second pot season were taken to be tested under the same salinity levels through *in vitro* tissues culture experiments.

MATERIALS AND METHODS

The present work was carried out in the Biotechnology and Bioengineering lab. as well as the plastic greenhouse of the Plant Physiology Division, Faculty of Agriculture, Cairo University, Giza, Egypt. This part including one *in vitro* tissue culture experiment which was conducted during the period from May, to October 2001. The grains produced by the plants of both used cultivars in the second pot experiment season [2000/2001] were used to be tested through this tissue culture experiment.

Preparation of culture media:

For preparation of 1 liter from the culture MS-medium (Murashige and Skooge, 1962) 4.40 g powder MS-medium was used in addition to the other components; sucrose 30 g/l, agar 8 g/l and 2 mg/l 2,4-D (2-4 dichlorophenoxy acetic acid) and the pH was adjusted to 5.8. The culture medium was sterilized by Russian autoclaving at 121 ^{0}C at (15 Psi for 30 min).

Embryo isolation and callus initiation:

The produced grains in first part by the plants of control I and control II as well as the different treatments were soaked for 12 hours in distilled water and surface sterilized for 30 min. with sodium hypochlorite (Clorox) and few drops from Tween 20 and rinsed 5 times with sterilized distilled water.

The embryos were excised aseptically under sterilized concentrations by using the American air laminar flow apparatus (NU.Air) and then were cultured in jars containing 50 ml agar solidified MS medium supplemented with 2 mg/l 2,4-D and 3% sucrose and adjusted to pH of 5.8. The embryos were cultured and incubated in a growth room at 25 ^{0}C ± 2 under 16 hours light and 8 hours darkness, using day light florescent lamps and supported with (40 ME- cm^{2}/S).

Salinity treatments for callus:

The produced calli for each treatment (6 weeks after culturing) were subdivided into one g inoculum pieces and subcultured on a solidified MS-medium + the same concentrations of 2,4-D and sucrose but supplemented with the different levels of salinity at the rate of 0.0%, 15%, 30% and 45% sea water and the pH was adjusted at 5.8. Jars of 300 ml in size, containing 50 ml of agar solidified MS-medium were used. The cultures were incubated for 6 weeks under the same previously mentioned conditions, under the different levels of salinity, the fresh weights of the growing calli were recorded after 2, 4 and 6 weeks, and 10 replicates were used for each treatment in this respect.

Plant regeneration media:

The calli (20 replicates) of the all tested treatments were subdivided into 500 mg inoculum pieces and subcultured on the "plant regeneration media" (the same media but supplemented with 1 mg/l zeatin riboside). After 4 weeks on plant regeneration media supplemented with the different levels

of salinity, the shoots were produced, then left to grow for another 4 weeks on the same media. At 8 weeks (4 + 4 weeks), the root formation was realized.

Acclimatization of the growing plantlets, plant growth and grain production:

The produced plantlets were washed with tape water for three times to remove all of agar traces, then soaked in Vita Fax 2.0 g/l for 1 min. and transferred to grow under plastic greenhouse conditions in black plastic pots containing an equal amount of the mixture of peatmoss and fine sand 1:1 (v/v) (Murali *et al.*, 1993), the pH of the peatmoss was adjusted to 6.0 by adding $CaCO_3$. The white fluorescent lamps which giving intensity of about 1500 lux for 16 hours per day were used. The temperature was 28°C and the relative humidity 85% by adding water every three hours for half hour through the mist, and the complete nutrient solution as described by Hewitt (1952) was added every three days. Irrigation of the growing plants was done by using the same mentioned levels of salinity. During the growth period, 15 shoots from each treatment were sampled after 45 days and their shoot heights were recorded, then the shoots were divided into two groups. The first one, i.e. 10 shoots were dried and their crude dry weights were determined, then prepared for nutrients, sugars and free amino acids estimations, while the second group, i.e. 5 shoots were kept as fresh materials for proline and invertase activity estimation as already mentioned in detail in the first part of this work. At harvest, i.e. after 2 months in pots, the grain yield (g)/plant and the weight of 1000 grains were recorded. The mature wheat grains of each treatment were powdered and prepared for chemical analyses as previously done in the first part of this work.

Data of calli fresh weights, growth parameters of the regenerated shoots and yield component were statistically analyzed and the mean values were compared using New L.S.D. values at 5% and 1% levels (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

a-Callus fresh weight:

The results in Table 1 clearly reveal that fresh weight of the embryonic wheat calli after 2, 4 and 6 weeks showed gradual highly significant decrease as the salinity level increased to reach the lowest values at the higher level of salinity; 45% sea water as compared with the fresh weight of the non-stressed control.

Decreasing the calli growth in response to increasing salinity level in the medium was previously reported by El-Shafey *et al.* (1994); El-Shihy *et al.* (1994 a&b); El-Hennawy (1996); Safarnejad (1996); Ahmed (1999) and Quraishi *et al.* (2000). On the other hand Yasuda *et al* (1982) reported that the growth of sugar can calli was inhibited by inclusion of different levels of NaCl on the culture medium and no plant regeneration was obtained.

On the other hand, when consider the mean values of calli fresh weights due to each treatment (Mean T) regardless the salinity level it could be noticed that, a similar trend to that previously described due the effect of the same treatments on the morphological characters and dry matter

accumulation of the stressed shoots and roots (in the first part, El-Shafey *et al.*, 2003a) can be drawn on the case of callus fresh weight. Hence, the all treatments resulted in increasing calli fresh weight, but the superiority was confirmed for the treatments of the weekly spraying either with 10 µM putrescine or 2 ppm ABA as well as grain irradiation with 15 KR gamma rays which yielded the highest highly significant increases in the calli fresh weight over the respective values of both sensitive and tolerant controls. Meanwhile, the other three treatments, i.e. 20 KR gamma rays, soaking in 2 ppm ABA or soaking + spraying with 2 ppm ABA recorded their highly significant increments over the respective values of the sensitive control only. This was true, either after 2, 4 or 6 weeks in culture, with clear superiority of putrescine treatment which yielded the highest highly significant increases in the mean value (Mean T) of calli fresh weight over the salt-tolerant control which were 19.0%, 18.7% and 20.4% after 2, 4 and 6 weeks, respectively, meanwhile the respective percentage of the same treatment at 30% sea water were 18.6%, 21.8% and 27.3% respectively. Comparing the effect of the interaction between treatments and salinity level clearly reveal that, although, no significant differences were recorded due to the interaction after 2 weeks, the recorded calli fresh weight values either after 4 or 6 weeks showed that the calli derived from the grains of the treatments of spraying with putrescine, 2 ppm ABA and 15 KR gamma rays recorded their highly significant increment over both controls up to 30% sea water, and only up to 15% sea water level for the other three treatments. Similar results were reported by El-Shihy *et al.* (1994 a) on the faba bean calli as affected by fast neutron and gamma rays treatments, as well as El-Shafey *et al.* (1998) on wheat calli as affected by the combination between 15 KR gamma rays + 5 mM sodium azide.

Table (1): Effect of salinity treatments on the calli fresh weights (g) derived from wheat embryos of the mature grains produced by the salt-sensitive Giza 167 wheat cultivar plants treated with gamma rays, abscisic acid and putrescine in comparison with those produced by the untreated salt-tolerant Sakha 8 cultivar plants under saline conditions (%sea water) during 2000-2001 season. Fresh weights were recorded after 2,4,and 6 weeks, the inoculum weight was 1.0 g.

Treatments / % sea water	0	15	30	45	Mean T	0	15	30	45	Mean T	0	15	30	45	Mean T
	2 Weeks					4 Weeks					6 Weeks				
Sakha 8 control	2.23	1.84	1.56	1.31	1.74	2.89	2.77	2.43	1.56	2.41	3.48	3.12	2.56	1.63	2.70
Giza 167 control	1.65	1.41	1.36	0.97	1.35	2.47	2.25	1.71	0.96	1.85	3.36	2.41	1.88	1.21	2.22
Gamma rays 15 KR	2.92	2.33	1.99	0.83	2.02	3.84	3.55	2.95	0.41	2.69	5.16	4.01	3.21	0.40	3.20
Gamma rays 20 KR	2.47	2.08	1.14	0.77	1.62	3.61	3.31	1.18	0.44	2.14	4.96	3.87	1.24	0.38	2.61
Soaking in 2 ppm ABA	2.44	2.12	1.12	0.78	1.62	3.46	3.34	1.20	0.42	2.11	4.89	3.88	1.26	0.37	2.60
Soaking + spraying with 2 ppm ABA	2.43	2.13	1.11	0.77	1.61	3.52	3.41	1.19	0.43	2.14	4.74	3.78	1.26	0.34	2.53
Weekly spraying with 2 ppm ABA	2.95	2.59	1.84	0.81	2.05	3.93	3.67	2.86	0.42	2.72	5.13	4.01	3.22	0.39	3.19
Weekly spraying with 10 µM putrescine	2.98	2.61	1.85	0.85	2.07	4.14	3.88	2.96	0.45	2.86	5.22	4.11	3.26	0.39	3.25
Mean (S)	2.51	2.14	1.50	0.89		3.48	3.27	2.06	0.64		4.62	3.65	2.24	0.64	
New LSD value at	0.05	0.01				0.05	0.01				0.05	0.01			
Salinity (S)	0.13	0.17				0.13	0.18				0.16	0.22			
Treatment (T)	0.18	0.24				0.19	0.25				0.23	0.31			
(S) X (T)	NS	NS				0.37	0.50				0.46	0.61			

It is worthy to point out here that, the all derived calli either from the both controls or the all other treatments were able to continue their growth even at the highest level of salinity, though with differential degrees, and were able to produced shoots under the all salinity level. In this concern, the treatments of the weekly spraying either 10 µM putrescine or 2 ppm ABA as well as 15 KR gamma rays were superior in recording the highest significant mean values of

calli fresh weight (Mean T) as well as the highest significant increases over both controls up to 30% sea water level. This clearly indicating that such growing calli showed superiority in their salinity tolerance under the *in vitro* conditions induced by the same three treatments, as did in the pot experiments in the first part of this work. Therefore, Yang *et al* (1990) concluded that callus growth and Na^+/K^+ ratio could be used as indicator of whole plant salt tolerance in sorghum.

b-Shoot height and dry weight of the regenerated plants:

The results in Table 2 represent the different values of plant height and shoot dry weight (g/plant) of regenerated 45 days-old plants which, irrigated with the same different levels of salinity (% sea water) under the green house conditions in the black plastic pots contained a mixture of peatmoss and fine sand 1:1 (v/v). Comparing the heights of the produced shoots from wheat calli of the different treatments when grown under different levels of salinity (% sea water), it is to be observed that the exact identity to the trend previously described in the pot experiments due the negative effects of salinity on shoot height as well as the positive effects of different applied treatments on the same parameter. Thus, the treatments of spraying with putrescine, 2 ppm ABA, and 15 KR gamma rays irradiation showed superiority in inducing the highly significant increments in the shoot height of the regenerated plants over both untreated controls, meanwhile the other three treatments recorded the highly significant increases in the mean values (Mean T) of shoot height only over the respective values of the salt-sensitive control. The absolute superiority in inducing the highest significant increments in the shoot height over the respective values of tolerant control was confirmed for putrescine treatments (23.0%). Meanwhile, although no significant differences were recorded due to the interaction between salinity levels and treatments, the same putrescine treatments recorded the highest considerable increases in the shoot height (40.4%) as compared with the tolerant control at 30% sea water, while the increases values due to the spraying with 2 ppm ABA and 15 KR gamma rays treatments were 31.0% and 29.1%, respectively, at 30% sea water level. As regards shoot dry weight of regenerated 45 day-old plants, the recorded data in Table 2, are in full accordance with those previously obtained due to the effects either of salinity or the different treatments on shoot dry weights of the potted plants in the first part of this study. Therefore, although the same expected negative correlation between salinity level and growth and dry matter accumulation of the regenerated shoots dry weight was recorded, on the contrary, the highest significant positive effects on increasing the dry matter accumulation of shoot still recorded with putrescine treatments (30.5%) followed by spraying with 2 ppm ABA (26.7%) then 15 KR gamma rays (21.9%) over the respective mean value (Mean T) of the salt tolerant Sakha 8 control. However, the other three treatments were highly significant exceeded the respective Mean T of the salt-sensitive control only. Moreover, the most effective treatments, i.e. weekly spraying either with putrescine, 2 ppm ABA as well as 15 KR gamma rays could highly significant surpassed the salt-tolerant control in this regard up to 30% sea water, meanwhile, the other three treatments surpassed it only up to 15% sea water level. Similar results were reported by El-Shafey *et al.* (1994) with wheat as

affected by the combination between 15 KR gamma rays + 5 mM sodium azide and with rice by 5 KR gamma rays + 1 mM sodium azide. In accord, also with the obtained results in the present work, Tal *et al.* (1977) found that a positive correlation between salt tolerance of the whole tomato plants and calli derived from different organs of these plants. Moreover, Nabors *et al.* (1980) stated that tobacco plants regenerated from salt-tolerant cells had a high survival rate in the presence of salt and the plants were reported to transmit tolerance to the generation.Meanwhile, Nabors and Dykes (1985) reported that more than 4000 plants have been regenerated from tissue culture derived from *in vitro* selected NaCl-tolerant cells of rice, wheat. In each case, the selected tolerant cells were heritable and stable in the regenerated plants. On the other hand, Whan *et al.* (1991) succeeded in producing two salt-resistant mutants of citrus after treating callus with gamma rays (5-8 KR) and ethyl methane sulfonate.

Table (2):Effect of salinity treatments on the plant height and shoot dry weight (g/plant) of the regenerated plants (45 days - old) produced from mature grains embryos of the salt-sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with those produced by untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

% sea water / Treatments	0	15	30	45	Mean T	0	15	30	45	Mean T
	Plant height					Shoot dry weight				
Sakha 8 control	30.56	27.19	22.34	15.23	23.83	1.52	1.14	0.93	0.62	1.05
Giza 167 control	27.15	22.22	17.43	12.60	19.85	1.35	0.87	0.66	0.41	0.82
Gamma rays 15 KR	37.22	32.64	28.83	10.22	27.23	2.01	1.68	1.35	0.08	1.28
Gamma rays 20 KR	34.65	30.83	15.02	10.33	22.71	1.89	1.52	0.39	0.07	0.97
Soaking in 2 ppm ABA	35.86	29.57	14.37	9.81	22.40	1.87	1.63	0.37	0.07	0.99
Soaking + spraying with 2 ppm ABA	36.58	30.87	13.34	9.48	22.57	1.88	1.56	0.38	0.06	0.97
Weekly spraying with 2 ppm ABA	38.56	33.38	29.27	10.30	27.88	2.12	1.88	1.22	0.09	1.33
Weekly spraying with 10 µM putrescine	40.20	35.36	31.37	10.30	29.31	2.18	1.96	1.24	0.10	1.37
Mean (S)	35.10	30.26	21.50	11.03		1.85	1.53	0.82	0.19	
New LSD value at		0.05	0.01				0.05	0.01		
Salinity (S)		1.24	1.64				0.07	0.09		
Treatment (T)		1.75	2.32				0.10	0.13		
(S) X (T)		NS	NS				0.19	0.26		

c- Chemical analyses of the generated plants:

Data in Table 3 concerning sugar concentrations in the shoots of the regenerated plant of 45 days-old grown under the different salinity levels, clearly showed the same general behavior and response that obtained with sugar concentrations in the shoots of the growing plants in pots in the first part as affected by the same treatments under the same salinity levels. Thus, the present results in Table 3 strongly supported the previously conclusion drawn in the pot experiments (in the second season) regarding the positive effects of increasing salinity level on sugar concentrations.

On the other hand, it is of great interest to observe that, the absolute superiority was confirmed here also for the treatments of spraying either with putrescine or 2 ppm ABA as well as 15 KR gamma rays in inducing the highest increases in the Mean T of concentrations of sugars (especially non-reducing ones) in the produced shoots tissues, especially those derived from the calli of the grains produced by the treated plants with putrescine which recorded the highest accumulation of sugars in the produced shoots tissues over the respective Mean T of the salt-tolerant control shoots, meanwhile, the other three treatments exceeded only the salt-sensitive control in this regard. Moreover, if the effect of the interaction between treatments and salinity level

was considered, it could be realized that the three super treatments accumulated much more concentrations of sugars which greatly exceeded both controls up to 30% sea water level, while the other three treatments exceeded the tolerant control up to 15% only. This finding is in full accordance with the conclusion previously drawn with sugar analyses in the shoots of potted plants under the same conditions in the first part of this investigation (El-Shafey *et al.*, 2003a). The obtained results are in accordance with those obtained by El-Shihy *et al.* (1994 a) with gamma rays and faba bean. Also, this finding represents great support for the opinion of Strogonov (1970) who concluded that under saline conditions the accumulation of non-toxic substances such as sucrose, proline, organic acid and protein is considered to be a protective adaptation and that the survival of plants under saline conditions depends upon the regulation of metabolic processes and the quantitative ratio between the protective and toxic metabolic intermediates. Similarly, Itoth and Kumora (1987) reported that both K^+ and sugars contribute to the osmotic adjustment in many plant species. Moreover, Gananasiri *et al.* (1990) suggested that osmoregulation in stressed plants was higher with K^+ and sugars. Both of them were found to correlate negatively with osmotic potential. In addition, Kirst (1990) considered sucrose as one of the compatible solutes that increase during salt stress. More recently, Pessarakli (2002) concluded that the plants that fail to increase soluble sugars biosynthesis could not tolerate salt. Moreover, it is well known that sugars as osmolytes enable plants to keep better water relation under water and salt stress conditions. This strongly suggesting an active protective mechanism in the produced shoots, induced by the most effective treatments, i.e. the weekly spraying either with putrescine or 2 ppm ABA as well as 15 KR gamma rays, in favor of increasing tolerance to salinity in the regenerated plants, as did with the parent plants grown in the pot experiments in the first part of this work. This also indicate that the tolerant plants were reported to transmit tolerance to their generation as previously concluded by Nabors *et al.* (1980).

Table (3):Effect of salinity treatments on the reducing, non-reducing and total sugars concentrations (mg glucose/g dry weight) in the shoots of the regenerated plants (45 days - old) produced from calli of the grains of the salt - sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % Sea water	Reducing sugars					Non-reducing sugars					Total sugars				
	0	15	30	45	Mean T	0	15	30	45	Mean T	0	15	30	45	Mean T
Sakha 8 control	27.65	30.05	38.01	42.78	34.62	29.99	31.38	40.09	49.16	37.66	57.64	61.43	78.10	91.94	72.28
Giza 167 control	21.33	26.01	35.11	37.01	29.87	26.45	29.70	36.18	40.51	33.21	47.78	55.71	71.29	77.52	63.08
Gamma rays 15 KR	31.85	33.16	40.06	41.16	36.56	34.52	36.31	45.92	47.16	40.98	66.37	69.47	85.98	88.32	77.54
Gamma rays 20 KR	30.16	31.33	33.01	35.49	32.50	32.51	34.11	35.22	37.84	34.92	62.67	65.44	68.23	73.33	67.42
Soaking in 2 ppm ABA	29.62	31.30	33.11	35.96	32.50	31.82	33.34	35.18	37.12	34.37	61.44	64.64	68.29	73.08	66.87
Soaking + spraying with 2 ppm ABA	29.42	31.28	33.46	35.42	32.40	30.67	33.22	35.07	37.90	34.22	60.09	64.50	68.53	73.32	66.62
Weekly spraying with 2 ppm ABA	31.42	35.11	40.77	41.82	37.28	34.19	37.27	46.11	47.66	41.31	65.61	72.38	86.88	89.48	78.59
Weekly spraying with 10 µM putrescine	33.74	35.34	40.02	41.72	37.71	35.14	38.92	46.68	47.78	42.13	68.88	74.26	86.70	89.50	79.84
Mean (S)	29.40	31.70	36.69	38.92		31.91	34.28	40.06	43.14		61.31	65.98	76.75	82.06	

As for proline concentrations in the regenerated shoots, data in Table 4 showed similar trend to that obtained in the shoots of the potted plants in the

first part with proline concentrations either due to the positive effect of salinity of proline concentration or the superiority of spraying with putrescine, 2 ppm ABA as well as 15 KR gamma rays in inducing considerable increases in the mean value (Mean T) of proline over the respective value of the salt tolerant control and up to 30% if the interaction between salinity and treatments was considered. This represents another supportive evidence of the increasing tolerance to salinity in the regenerated shoot, since, the endogenous concentrations of free proline in the stressed plants can be used as an indicator of salt tolerance. For each plant, this appears to be an external salt concentration above which, the plant's proline level sharply rises. This critical point is directly related to the ability of plant to tolerate salt. Thus, measurements of proline concentrations can be used to determine salt resistance of plant. In *Sorghum bicolor* leaves, proline did not start to accumulate until the concentration of total monovalent cations reached a three fold of approximately 200µmol/g fresh weight (Weimberg *et al.*, 1982). Moreover, the accumulated proline in *Aster tripolium* plants decrease quickly when plants were transferred to a medium without NaCl (Goes and Larther, 1982). Evidently, the main feature of increasing salinity concentration in the plant medium is the accumulation of proline in the stressed tissues.

The data of invertase activity in the regenerated shoots of 45 day-old in Table 4 clearly showed the same constant trend that obtained and described in the case of stressed leaves of the growing wheat plants in the pot experiments in the first part of this work, that is, the negative correlation between increasing salinity level and invertase activity, was also more pronounced as a result of spraying with putrescine, 2 ppm ABA and 15 KR gamma rays irradiation which recorded much lower activities as compared with that in the salt-tolerant control at the all applied levels of salinity as well as when calculating the mean value of each treatment (Mean T), the only exception is that, at 45% sea water when the enzyme activity in the treated plants was nearly equal to its activity in the salt-tolerant control. On the contrary, the other three treatments, i.e. 20 KR gamma rays, soaking in 2 ppm ABA and soaking + spraying with 2 ppm ABA increased invertase activity over that in the salt-tolerant control at 15%, 30% and 45% sea water and consequently the mean values of these treatments (Mean T) corresponded to the same trend. This finding added another supportive evidence for the superiority of spraying with putrescine, 2 ppm ABA and 15 KR gamma rays treatments in increasing salinity tolerance of the regenerations brought about by inhibiting invertase activity in favor of accumulations of more quantities of non-reducing sugars, which play the vital role in improving salinity tolerance of the regenerated *in vitro* plant. This finding also strongly indicating that the high degree of tolerance to salinity in the treated-sensitive wheat plants, was transmissible to the regenerations under *in vitro* conditions. Also, this finding is in full accordance with the conclusion drawn by Nabors *et al.* (1980) who concluded that plants regenerated from salt-tolerant cells had a higher survival rate in the presence of salt, and the plants were reported to transmit tolerance to the generations.

The concentration of free amino acids in the regenerated shoot in *in vitro* tissue culture experiments (Table 5) showed nearly similar trend to that

recorded in the treated shoots in the first part of this investigation as regards the positive correlation between increasing salinity level as amino acids concentrations. In addition, the treatments of spraying either with putrescine or 2 ppm ABA as well as grains irradiation with 15 KR gamma rays induced their considerable increases in amino acid concentrations over both controls only up to 30% sea water, in addition to exceeding the mean values of concentrations of the salt tolerant control (Mean T). Meanwhile, the other three treatments exceeded only the Mean T of the salt sensitive control and surpassed the salt-tolerant one only up to 15% sea water level.

Table (4):Effect of salinity treatments on the proline concentration (mg/g fresh weight) and invertase activity (µmol glu/ min.g fresh weight) in the shoots of the regenerated plants (45 days- old)produced from calli of the grains of the salt-sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % Sea water	Proline					Invertase				
	0	15	30	45	Mean T	0	15	30	45	Mean T
Sakha 8 control	2.53	2.97	4.88	5.93	4.08	66.34	41.05	30.16	18.40	38.99
Giza 167 control	1.38	1.51	3.82	4.07	2.70	69.10	53.33	43.51	28.40	48.59
Gamma rays 15 KR	3.96	4.93	5.09	5.14	4.78	54.46	34.26	26.38	21.31	34.10
Gamma rays 20 KR	2.98	3.49	3.69	3.96	3.53	59.64	48.69	38.39	28.54	43.82
Soaking in 2 ppm ABA	2.86	3.58	3.71	3.98	3.53	61.44	49.29	39.47	27.56	44.44
Soaking + spraying with 2 ppm ABA	2.95	3.48	3.77	3.96	3.54	63.14	46.34	38.65	25.36	43.37
Weekly spraying with 2 ppm ABA	4.14	4.73	5.14	5.22	4.81	50.33	35.41	24.22	21.42	32.85
Weekly spraying with 10 µM putrescine	4.19	4.88	5.17	5.36	4.90	48.77	32.30	23.41	19.12	30.90
Mean (S)	3.12	3.70	4.41	4.70		59.15	42.58	33.02	23.76	

The recorded data in Table 5 clearly reveal that the same constant behavior and trend of response that previously described due to the negative effect of salinity as well as the positive effect of the different treatments on crude protein concentrations in the growing treated shoots in the pot experiments in the first part can be drawn here in the case of regenerated shoots under *in vitro* conditions (Table 5) with special referring to the absolute superiority of the weekly spraying with 10 µM putrescine treatment which recorded the highest increments in protein concentrations (Mean T) as well as up to 30% sea water over the respective values of the salt-tolerant control followed by spraying with 2 ppm ABA then the grain irradiation with 15 KR gamma rays.

Table (5): Effect of salinity treatments on the free amino acids and crude protein concentrations (mg/g dry weight) in the shoots of the regenerated plants (45 days - old) produced from calli of the grains of the salt-sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % Sea water	Free amino acids					Crude protein				
	0	15	30	45	Mean T	0	15	30	45	Mean T
Sakha 8 control	4.77	6.99	10.19	14.75	9.18	138.19	128.38	107.56	78.38	113.13
Giza 167 control	3.73	4.89	9.57	10.18	7.09	125.13	109.63	97.00	59.44	97.80
Gamma rays 15 KR	6.11	8.86	12.26	13.12	10.09	168.75	153.25	125.06	55.63	125.67
Gamma rays 20 KR	5.91	7.96	8.21	9.41	7.87	156.44	147.13	81.38	53.63	109.64
Soaking in 2 ppm ABA	5.97	7.86	8.32	9.72	7.97	156.50	140.88	75.19	53.88	106.61
Soaking + spraying with 2 ppm ABA	5.88	7.88	8.18	9.63	7.89	162.69	138.50	68.81	44.44	103.61
Weekly spraying with 2 ppm ABA	6.36	8.63	12.85	13.13	10.24	187.69	165.69	133.31	55.13	135.45
Weekly spraying with 10 µM putrescine	6.39	8.91	13.10	13.76	10.54	197.38	168.75	138.44	55.69	140.06
Mean (S)	5.64	7.75	10.34	11.71		161.59	144.02	103.34	57.02	

Regarding N, P and K^+ concentrations in the regenerated shoots of 45 days-old plants, the results in Table 6 showed that N, P and K^+ concentrations in the regenerated shoots tended to decreased gradually by

increasing salinity level in the medium to reach their lowest values at the highest level of salinity, i.e. 45% sea water level. As for the spraying with putrescine, 2 ppm ABA and irradiation with 15 KR gamma rays, their superiority was confirmed here also, that is, the only appreciable increases over the Mean T over the salt-tolerant control of nutrient concentrations were recorded by these three treatments, which also exceeded the salt-tolerant control in their concentrations of N, P and K^+ up to 30% sea water level, meanwhile the other three treatments exceeded it only up to 15% sea water level.

As for Ca^{+2} and Mg^{+2} concentrations in the regenerated shoots, the recorded data in Table 6 clearly showed that a similar trend to that previously obtained due to the effect of salinity on Ca^{+2} and Mg^{+2} in the growing shoots in the pot experiments can be drawn in the case of regenerated shoots, regarding the positive correlation between Ca^{+2} and Mg^{+2} concentrations and increasing salinity levels. Moreover, when detecting the effect of different treatments on Ca^{+2} and Mg^{+2} levels, it could be realized that for both nutrient concentrations, the treatments of putrescine, spraying with 2 ppm ABA and 15 KR gamma rays were also superior in the all studied cases either in their exceeding the salt-tolerant control up to 30% sea water level or in the Mean T for Ca^{+2} and Mg^{+2} concentrations, meanwhile the other three treatments could only exceeded the Mean T of the salt-sensitive control and could exceeded the tolerant one only up to 15% sea water level.

The most interesting and important comparison is that between both tolerant and sensitive control which clearly indicate that, as did in the first part, the salt-sensitive Giza 167 control shoots contained much lower quantities of the all estimated components than that the salt-tolerant one, except only with invertase activity and Na^+ concentrations, when the reverse trend was recorded.

Table (6): Effect of salinity treatments on the $N, P, K^+, Na^+, Ca^{+2}, Mg^{+2}$ concentrations (mg /g dry weight) in the shoots regenerated plants (45 days - old) produced from calli of embryos of mature grains of the salt-sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % Sea water	0	15	30	45	Mean T	0	15	30	45	Mean T	0	15	30	45	Mean T
	N					P					K				
Sakha 8 control	22.11	20.54	17.21	12.54	18.10	2.64	2.42	2.14	1.39	2.15	25.14	21.25	19.63	15.77	20.45
Giza 167 control	20.02	17.54	15.52	9.51	15.65	2.02	2.05	1.86	1.03	1.74	22.82	18.91	17.43	12.64	17.95
Gamma rays 15 KR	27.00	24.52	20.01	8.90	20.11	3.19	2.98	2.64	0.95	2.44	29.23	28.33	24.81	10.72	23.27
Gamma rays 20 KR	25.03	23.54	13.02	8.58	17.54	3.02	2.82	1.69	0.88	2.10	26.84	23.62	16.43	10.31	19.30
Soaking in 2 ppm ABA	25.04	22.54	12.03	8.62	17.06	3.04	2.87	1.59	0.82	2.08	27.25	24.11	16.63	10.31	19 58
Soaking + spraying with 2 ppm ABA	26.03	22.16	11.01	7.11	16.58	3.06	2.89	1.44	0.79	2.05	26.36	24.32	16.42	10.56	19.42
Weekly spraying with 2 ppm ABA	30.03	26.51	21.33	8.82	21.67	3.16	2.99	2.84	0.95	2.49	32.18	28.17	23.41	10.82	23.65
Weekly spraying with 10 µM putrescine	31.58	27.00	22.15	8.91	22.41	3.19	3.10	2.89	0.99	2.54	33.92	29.18	26.16	10.91	25.04
Mean (S)	25.86	23.04	16.54	9.12		2.92	2.77	2.14	0.98		27.97	24.74	20.12	11.51	
	Na					Ca					Mg				
Sakha 8 control	2.19	2.65	2.74	3.15	2.68	5.14	7.17	11.02	12.99	9.08	2.88	2.99	5.51	7.15	4.63
Giza 167 control	2.37	2.71	3.42	4.15	3.16	3.31	5.02	9.95	10.96	7.31	2.05	2.64	4.11	5.36	3.54
Gamma rays 15 KR	1.97	2.26	2.58	4.33	2.79	6.85	9.96	12.22	12.64	10.42	3.14	3.87	6.14	6.70	4.96
Gamma rays 20 KR	2.01	2.33	4.55	5.87	3.69	6.31	8.99	9.16	9.04	8.38	3.05	3.77	3.91	4.16	3.72
Soaking in 2 ppm ABA	2.07	2.38	4.87	5.77	3.77	6.29	8.84	9.08	9.57	8.45	3.06	3.73	3.98	4.18	3 74
Soaking + spraying with 2 ppm ABA	2.06	2.36	4.98	5.31	3.68	6.25	8.88	9.11	9.69	8.48	3.09	3.72	3.95	4.19	3.74
Weekly spraying with 2 ppm ABA	1.99	2.22	2.54	4.31	2.77	6.91	9.97	12.39	12.75	10.51	3.24	3.93	6.16	6.89	5.06
Weekly spraying with 10 µM putrescine	1.96	2.18	2.54	4.30	2.75	6.99	9.99	12.45	12.79	10.56	3.37	4.05	6.24	6.96	5.16
Mean (S)	2.08	2.39	3.53	4.65		6.01	8.60	10.67	11.30		2.99	3.59	5.00	5.70	

The recorded data in Table 6 clearly showed that Na^+ concentrations corresponded to the same previously obtained trend in the first part of this work due to increasing salinity level, though, with slightly higher values in the regenerated shoot. Moreover, when considered the mean values of each treatments (Mean T) of Na^+ concentration it could be noticed that, the shoots of putrescine, 2 ppm ABA and 15 KR gamma rays treatments showed nearly equal mean values of concentrations compared to the salt-tolerant control, meanwhile, Na^+ concentrations in the other three treatments exceeded the respective Mean T of Na^+ concentrations in both controls. On the other hand, although, the three super treatments showed lower Na^+ values than both controls up to 30% sea water and nearly similar values to that of the salt-sensitive one at 45% level, meanwhile the other three treatments, i.e. 20 KR gamma rays, soaking in 2 ppm ABA and soaking + spraying with 2 ppm ABA exceeded both controls at 30% and 45% sea water level.

Nevertheless, K^+/Na^+ and Na^+/Ca^{+2} ratios in Table 7 exhibited the same trend that previously recorded with the growing shoots in the pot experiments, so that, in spite of the negative effects of increasing salinity levels on the K^+/Na^+ ratio, the treatments of the weekly spraying either with putrescine or 2 ppm ABA as well as grain irradiation with 15 KR gamma rays were superior in recording the highest values of K^+/Na^+ ratio, i.e. 10.88, 10.15 and 9.87, respectively which greatly exceeded the Mean T of both controls, i.e. 7.92 and 6.19 for tolerant and sensitive ones, respectively as well as the higher individual values over both controls up to 30% sea water levels, i.e. K^+/Na^+ ratio recorded by the same putrescine, spraying with 2 ppm ABA and 15 KR gamma rays at 30% sea water were 10.30, 9.22 and 9.62, respectively meanwhile, the comparable ratios for tolerant and sensitive control at the same 30% level were 7.16 and 5.10 respectively. Meanwhile, the other three treatments exceeded only the Mean T of the salt-sensitive control and exceeded both control up to 15% sea water only. This strongly indicating the superiority of spraying with putrescine, 2 ppm ABA and 15 KR gamma rays in inducing more tolerance to salinity even in the regeneration which produced from the treated plants. Since, Yang *et al.* (1990) concluded that Na^+/K^+ ratio and callus growth could be used as indicators of sorghum whole plant salt tolerance. As regards, Na^+/Ca^{+2} (Table 7) and as did with shoots of the potted plants, in spite of the positive effect of the highest two levels of salinity, i.e. 30% and 45% sea water levels on Na^+/Ca^{+2}, the obtained values strongly confirmed the superiority of the same super treatments in inducing the lowest mean value (Mean T), i.e. 0.26, 0.26 and 0.27 compared with 0.32 and 0.49 for both tolerant and sensitive controls, respectively, as well as 0.42, 0.43 and 0.43 for the Mean T of other three treatments, in addition to the lowest values of Na^+/Ca^{+2} ratio under the all salinity levels up to 30% sea water, at which they recorded by the same super treatments, i.e. 0.20, 0.21 and 0.21 compared with 0.25 and 0.34 for both tolerant and sensitive controls as well as 0.55, 0.54 and 0.50 recorded by the other three treatments. This clearly indicate that the regenerated shoots were characterized by higher accumulation of N, P, K^+, Ca^{+2}, Mg^{+2}, proline, protein, sugars and free amino acids, in addition to the lowest quantities of Na^+ compared to both controls up to 30% sea water level. In the all of these cases the superiority of spraying

with either putrescine, 2 ppm ABA and 15 KR gamma rays was confirmed in inducing the highest degree of adaptation in the regenerated plants due to increasing the physiological tolerance that brought about by accumulation of both inorganic osmotica, i.e. ions as well as the organic osmolytes, i.e. sugars, proline, amino acids and protein.

Table (7): Effect of salinity treatments on the K^+/Na^+ and Na^+/Ca^{+2} ratios in the shoots of the regenerated plants (45 days-old) produced from calli of embryos of the mature grains the salt-sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % Sea water	K/Na					Na/Ca				
	0	15	30	45	Mean T	0	15	30	45	Mean T
Sakha 8 control	11.48	8.02	7.16	5.01	7.92	0.43	0.37	0.25	0.24	0.32
Giza 167 control	9.63	6.98	5.10	3.05	6.19	0.72	0.54	0.34	0.38	0.49
Gamma rays 15 KR	14.84	12.54	9.62	2.48	9.87	0.29	0.23	0.21	0.34	0.27
Gamma rays 20 KR	13.35	10.14	3.61	1.76	7.21	0.32	0.26	0.50	0.65	0.43
Soaking in 2 ppm ABA	13.16	10.13	3.41	1.79	7.12	0.33	0.27	0.54	0.60	0.43
Soaking + spraying with 2 ppm ABA	12.80	10.31	3.30	1.99	7.10	0.33	0.27	0.55	0.55	0.42
Weekly spraying with 2 ppm ABA	16.17	12.69	9.21	2.51	10.15	0.29	0.22	0.21	0.34	0.26
Weekly spraying with 10 µM putrescine	17.31	13.39	10.30	2.54	10.88	0.28	0.22	0.20	0.34	0.26
Mean (S)	13.59	10.52	6.46	2.64		0.37	0.30	0.35	0.43	

The specific effects of Na^+/Ca^{+2} ratio was recorded by several workers. It is evidenced that the adequate level of Ca^{+2} in the plant has beneficial effect for reduction Na^+ uptake, thus, plants with high Ca^{+2}/Na^+ ratio had more ability to exclude Na^+ under saline conditions (Kent and Lauchi, 1985). Also, the low Na^+/Ca^{+2} ratio is important in maintaining membrane function as reported by Greenway and Munns (1980) who added that, growth of beans was markedly influenced by the Na^+/Ca^{+2} ratio, at high external NaCl. Growth of beans decreased and Na^+ increased in the leaves only when Na^+/Ca^{+2} exceeded 17. Moreover, Hue and McCall (1989) found that in *Maca domia* seedlings, when plant K^+/Na^+ ratio was 2.5, adverse effects of salinity could be expected. K^+/Na^+ ratio of 1.5 is corresponding to 50% reduction in growth. Moreover, Hu and Cramer (1993) reported that growth of *Brassica carinata*, was significantly positively correlated with Ca^{+2} concentration as well as K^+/Na^+ and Ca^{+2}/Na^+ and negatively with Na^+ under saline conditions. Recently, Salem et al. (2002) found that the most salt-tolerant faba bean cultivars, i.e. Giza 429 and Giza 843 showed much higher Ca^{+2} and Mg^{+2} concentrations as well as K^+/Na^+ ratio and the lowest Na^+ quantities as well as the lowest Na^+/Ca^{+2} ratio compared with the other sensitive two cultivars, i.e. Giza 3 and Giza 674. On the other hand, depending on the response of K^+, Na^+ and Ca^{+2} level to increasing salinity, it seems, therefore, logic to expect that K^+/Na^+ ratio must be decreased and Na^+/Ca^{+2} allow the opposite pattern in their response to increasing salinity levels. Thus, and because the higher Na^+/K^+ in saline soils, Epstein (1972) stated that the salt-tolerant plants such as halophytes or those glycophytes which treated with specific treatments that induce such tolerance (as did in the present work) must develop a mechanism for preferential uptake of K^+ from mixture rich in Na^+. These plants must have a very develop "absorption system".

d- Chemical analyses of the produced grains:

Comparing sugar concentrations in the produced grains from the regenerated plants grown for two months under greenhouse conditions and irrigation with the same 0.0%, 15%, 30% and 45% sea water levels (Table 8)

clearly reveal that, the same trend and response of sugar concentrations to either increasing salinity levels or to the various treatments in the *in vitro* regenerated shoots (Table 3) can be also drawn in the case of the produced grains (Table 8). In the produced grains, the superiority of spraying with putrescine, 2 ppm ABA and irradiation with 15 KR gamma rays, was more pronounced here over the salt tolerant control up to 30% sea water, however, sugar concentrations in the grains produced from these treated plants were nearly equal to that in the grains of untreated salt-tolerant control at 45% sea water level. Thus, the same super three treatments greatly exceeded both controls when the Mean T was considered, for reducing, non-reducing and total sugars. Meanwhile, the other three treatments showed lower values than both controls at 30% and 45% sea water level. It is to be observed here also and as did with sugars in the all previous cases in both part of this work, the concentration of non-reducing sugars considerably exceeded that of reducing ones under the same conditions. This can be realized by comparing the concentrations of both sugars in the produced grains at 30% sea water level, which disclosed that the amount of non-reducing sugar was more than four or even five folds that of reducing sugars.

When comparing the concentrations of crude protein in the *in vitro* produced grains (Table 9) one could realize that the same response and constant trend that found and described with protein concentrations in the produced grains from the treated plant in the pot experiments in the first part (El Shafey *et al.*, 2003a) and that obtained in the regenerated shoots (Table 5) can be also drawn in the case of the produced grains from the regenerated plants (Table 9).

Table (8): Effect of salinity treatments on the reducing, non-reducing and total sugars concentrations (mg glucose/g dry weight) in the grains produced from calli of embryos of the mature grains produced by the salt-sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with those produced by the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % Sea water	Reducing sugars					Non-reducing sugars					Total sugars				
	0	15	30	45	Mean T	0	15	30	45	Mean T	0	15	30	45	Mean T
Sakha 8 control	2.86	4.16	8.11	10.22	6.34	23.99	28.06	31.58	37.47	30.28	26.85	32.22	39.69	47.69	36.61
Giza 167 control	2.42	3.11	6.13	7.28	4.74	20.15	24.12	32.31	33.91	27.62	22.57	27.23	38.44	41.19	32.36
Gamma rays 15 KR	4.21	5.97	9.33	9.85	7.34	28.91	32.52	36.11	36.67	33.55	33.12	38.49	45.44	46.52	40.89
Gamma rays 20 KR	3.93	5.18	5.81	6.96	5.25	26.55	30.14	31.86	32.18	30.18	30.48	35.32	37.67	38.14	35.43
Soaking in 2 ppm ABA	3.98	5.11	5.88	6.07	5.26	26.52	30.20	31.83	32.20	30.19	30.50	35.31	37.71	38.27	35.45
Soaking + spraying with 2 ppm ABA	3.87	5.19	5.86	6.10	5.26	26.03	30.16	31.73	32.31	30.06	29.90	35.35	37.59	38.41	35.31
Weekly spraying with 2 ppm ABA	4.22	6.09	9.40	9.91	7.41	29.56	32.71	36.22	37.03	33.88	33.78	38.80	45.62	46.94	41.29
Weekly spraying with 10 µM putrescine	4.29	6.11	9.50	9.97	7.47	30.64	32.93	36.30	37.08	34.24	34.93	39.04	45.80	47.05	41.71
Mean (S)	3.72	5.12	7.50	8.18		26.54	30.11	33.49	34.86		30.27	35.22	41.00	43.04	

Table (9): Effect of salinity treatments on the crude protein concentration and free amino acids (mg/g dry weight) in the grains produced from calli of embryos of the mature grains produced by the salt-sensitive Giza 167 wheat cultivar plants treated with gamma rays ·abscisic acid and putrescine in comparison with those produced by the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % Sea water	Free amino acids					Crude protein				
	0	15	30	45	Mean T	0	15	30	45	Mean T
Sakha 8 control	0.96	1.77	2.37	3.61	2.18	123.13	95.13	85.00	71.31	93.64
Giza 167 control	0.87	0.93	2.18	2.73	1.68	103.75	77.56	66.38	46.31	73.50
Gamma rays 15 KR	2.17	2.28	2.58	2.61	2.41	144.75	119.44	105.00	41.38	102.64
Gamma rays 20 KR	1.28	1.93	2.10	2.34	1.91	138.19	108.50	61.25	34.50	85.61
Soaking in 2 ppm ABA	1.41	2.06	2.13	2.36	1.99	140.63	116.38	61.88	35.00	88.47
Soaking + spraying with 2 ppm ABA	1.31	1.97	2.16	2.31	1.94	141.25	120.13	61.13	36.19	89.67
Weekly spraying with 2 ppm ABA	2.12	2.37	2.55	2.63	2.42	153.75	133.88	105.63	39.00	108.06
Weekly spraying with 10 µM putrescine	2.18	2.58	2.61	2.69	2.52	157.50	138.75	110.00	41.31	111.89
Mean (S)	1.54	1.99	2.34	2.66		137.87	113.72	82.03	43.13	

On the other hand, in spite of that free amino acid concentrations were positively affected by increasing salinity level, however, and as did with protein concentrations in the grains produced in the both first and second parts, the only increments in free amino acids concentrations over both controls at 30% sea water level of salinity as well as Mean T, were recorded in the produced grains only as a result of spraying with putrescine, 2 ppm ABA and 15 KR gamma rays treatments, which represents another supportive evidence for the superiority of these treatments (Table 9).

When comparing nutrient concentrations in the produced grains (Table 10), it could be easily realized that, N, P and K^+ concentrations exhibited the same behavior and response either negatively to increasing to salinity levels or positively to the various treatments, either in these produced grains and the regenerated shoots of the same plants (Tables 10 and 6 respectively) or in the grains produced from the parent treated plants in the first part of this study. Thus, the same trend can be, certainly, drawn in the all of these cases, with the same special referring to the superiority of spraying with putrescine, 2 ppm ABA and irradiation with 15 KR gamma rays treatments. Moreover, it is worth mentioning that, the superiority of these treatments was more pronounced when detecting Mg^{+2} and Ca^{+2} concentrations in the produced grains under *in vitro* conditions. The obtained data in Table 10 clearly reveal that the only marked increases in Ca^{+2} and Mg^{+2} concentrations over both controls values up to 30% and over the sensitive one at 45% sea water level were recorded only by the previous mentioned super treatments, thus, they greatly exceeded the Mean T of both controls, meanwhile the other three treatments failed to approach both controls values at 30% and 45% sea water levels in this regard.

Table (10): Effect of salinity treatments on the N,P, K^+ ,Na^+,Ca^{+2},Mg^{+2} concentrations (mg / g dry weight) in the grains produced from calli of embryos of mature grains of the salt-sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments / % Sea water	0	15	30	45	Mean T	0	15	30	45	Mean T	0	15	30	45	Mean T
	N					P					K				
Sakha 8 control	19.70	15.22	13.60	11.41	14.98	1.72	1.51	1.37	1.04	1.41	3.63	3.19	2.91	2.41	3.04
Giza 167 control	16.60	12.41	10.62	7.41	11.76	1.42	1.24	1.11	0.87	1.16	3.14	2.88	1.98	1.41	2.35
Gamma rays 15 KR	23.16	19.11	16.80	6.62	16.42	2.08	1.92	1.71	0.73	1.61	4.53	4.13	3.28	1.01	3.24
Gamma rays 20 KR	22.11	17.36	9.80	5.52	13.70	2.01	1.76	0.94	0.66	1.34	4.07	3.38	1.31	0.84	2.40
Soaking in 2 ppm ABA	22.50	18.62	9.90	5.60	14.16	2.01	1.81	0.92	0.64	1.35	3.96	3.40	1.40	0.94	2.43
Soaking + spraying with 2 ppm ABA	22.60	19.22	9.78	5.79	14.35	2.03	1.74	0.96	0.62	1.34	4.02	3.49	1.33	0.92	2.44
Weekly spraying with 2 ppm ABA	24.60	21.42	16.90	6.24	17.29	2.01	1.99	1.87	0.78	1.66	4.48	4.08	3.27	1.05	3.22
Weekly spraying with 10 µM putrescine	25.20	22.20	17.60	6.61	17.90	2.22	2.01	1.91	0.80	1.74	5.10	4.50	3.44	1.07	3.53
Mean (S)	22.06	18.20	13.13	6.90		1.94	1.75	1.35	0.77		4.12	3.63	2.37	1.21	
	Na					Ca					Mg				
Sakha 8 control	0.76	1.21	1.32	1.45	1.19	1.71	2.36	4.88	6.92	3.97	2.73	2.96	4.32	6.31	4.08
Giza 167 control	0.85	1.34	1.46	1.55	1.30	1.28	1.62	3.17	4.43	2.63	1.13	2.44	3.71	4.16	2.86
Gamma rays 15 KR	0.48	0.96	1.28	1.56	1.07	2.33	2.96	5.43	5.82	4.14	2.96	3.21	5.79	5.96	4.48
Gamma rays 20 KR	0.74	1.07	1.59	1.64	1.26	2.12	2.81	3.01	3.76	2.93	2.87	3.11	3.58	3.91	3.37
Soaking in 2 ppm ABA	0.72	1.15	1.56	1.69	1.28	2.22	2.89	3.04	3.71	2.97	2.95	3.04	3.51	3.89	3.35
Soaking + spraying with 2 ppm ABA	0.73	1.16	1.58	1.61	1.27	2.29	2.79	3.09	3.61	2.95	2.96	3.17	3.49	3.86	3.37
Weekly spraying with 2 ppm ABA	0.46	0.97	1.22	1.36	1.00	2.71	3.26	5.51	5.93	4.35	3.21	3.41	5.82	5.95	4.60
Weekly spraying with 10 µM putrescine	0.39	0.89	1.14	1.34	0.94	2.93	3.32	5.64	5.99	4.47	3.50	3.56	5.99	6.09	4.79
Mean (S)	0.64	1.09	1.39	1.53		2.20	2.75	4.22	5.02		2.79	3.11	4.53	5.02	

As regards Na^+ concentrations in the produced grains (also, Table 10), the obtained data clearly reveal that although the positive relation between Na^+ level and increasing salinity level, the superiority of the most effective

three treatments in lowering its concentrations to the lowest levels as compared to the all other treatments at the all applied salinity level up to 45% sea water one as well as in the Mean T was confirmed. On the other hand, it is worthy drawing attention to the most interesting comparison between Na^+ level in the produced grains (Table 10) and its respective concentrations in the *in vitro* regenerated shoots (Table 6) which clearly reveal that Na^+ concentration was gradually declined from the regenerated shoot tissues to attain its minimum levels in the produced grain especially as a result of the most effective treatments. This can be realized by comparing Na^+ concentrations due to these three treatments in the regenerated shoot tissues at the highest level of salinity, i.e. 45% sea water which were, 4.30, 4.31 and 4.33 mg/g dry weight (Table 6) and the respective concentrations in the produced grains (Table 10) which were, 1.34, 1.36 and 1.56 mg/g dry weight for the treatment of the weekly spraying putrescine, spraying with 2 ppm ABA and irradiation with 15 KR gamma rays, respectively. Accordingly, the percentages of reductions in Na^+ were, 68.8%, 68.4% and 64.0% respectively. Nevertheless, and due to the slightly lower concentrations of K^+ due to the same treatments at 45% sea water level, the same above mentioned super treatments recorded the highest values of K^+/Na^+ ratio, (3.02, 2.68 and 2.56) only up to 30% level compared with 2.20 and 1.36 for both tolerant and sensitive controls, respectively (Table 11) and 0.84, 0.90 and 0.82 for the other three treatments in spite of the sever negative effect of increasing salinity level on this ratio. On the contrary, the lowest Na^+/Ca^{+2} ratios, (0.20, 0.22 and 0.24) were recorded at 30% sea water levels by spraying with putrescine, 2 ppm ABA and 15 KR gamma rays, respectively compared with 0.27 and 0.46 for both tolerant and sensitive controls as well as 0.51, 0.51 and 0.53 for the other three treatments at the same salinity levels. Meanwhile, at 45 % sea water level the most effective three treatments values of Na^+/Ca^{+2} ratio were nearly equal to that of the salt-tolerant control (Table 11), thus, could record also the lowest Mean T values in this regard as compared the respective Mean T values of both controls.

Table (11): Effect of salinity treatments on the K^+/Na^+ and Na^+/Ca^{+2} ratios in the grains produced from calli of embryos of the mature grains the salt-sensitive Giza 167 wheat cultivar plants treated with Gamma rays, abscisic acid and putrescine in comparison with the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % Sea water	K/Na					Na/Ca				
	0	15	30	45	Mean T	0	15	30	45	Mean T
Sakha 8 control	4.78	2.64	2.20	1.66	2.82	0.44	0.51	0.27	0.21	0.36
Giza 167 control	3.69	2.15	1.36	0.91	2.03	0.66	0.83	0.46	0.35	0.58
Gamma rays 15 KR	9.44	4.30	2.56	0.65	4.24	0.21	0.32	0.24	0.27	0.26
Gamma rays 20 KR	5.50	3.16	0.82	0.51	2.50	0.35	0.38	0.53	0.44	0.42
Soaking in 2 ppm ABA	5.50	2.96	0.90	0.56	2.48	0.32	0.40	0.51	0.46	0.42
Soaking + spraying with 2 ppm ABA	5.51	3.01	0.84	0.57	2.48	0.32	0.42	0.51	0.45	0.42
Weekly spraying with 2 ppm ABA	9.74	4.21	2.68	0.77	4.35	0.17	0.30	0.22	0.23	0.23
Weekly spraying with 10 µM putrescine	13.08	5.06	3.02	0.80	5.49	0.13	0.27	0.20	0.22	0.21
Mean (S)	7.15	3.43	1.80	0.80		0.33	0.43	0.37	0.33	

e-Yield components:

For a certainly the most interesting and important feature of the obtained results in the *in vitro* tissue culture experiment is that the productivity of the regenerated plants, i.e. grain yield (g)/plant and the weight of 1000 grains, grown under the same salinity levels in the irrigation water,

nevertheless, without applying any treatments on these regenerated plants depending only on those previously applied on the parent plants in the first part of this study. The exact identity of both obtained trends of response of Giza 167 wheat plant productivity in both parts of this study under the same salinity levels, i.e. either of those treated in the pot experiments in the first part (El-Shafey et al., 2003a) or of the regenerated untreated plants under *in vitro* conditions in this second one, is of significant importance from both academic as well as applied point of view. This can be realized by comparing the recorded data in Table 12 with those previously obtained in the first part of this study. This comparison clearly reveal that the same trend that obtained as regards grain yield (g)/plant and the weight of 1000 grains (g) due to the effect of different treatments in the second season of the pot experiments in the first part, can be drawn in the case of the same parameters in the productivity of the regenerated plants under *in vitro* conditions (Table 12), though with lower values, which is quite expected.

For an interesting comparison, the percentage of the highly significant increases in grain yield (g)/plant over the comparable yield of the salt tolerant control due to putrescine, 2 ppm ABA and 15 KR gamma rays in both the two parts of this work (A and B) were compared. These percentage of increases in the case of the pot experiments (in the second season of the first part) at 30% sea water level were, 53.0%, 44.4% and 38.4% induced by putrescine, spraying with 2 ppm ABA and 15 KR gamma rays, respectively, meanwhile, the comparable values in the second one (Table 12) were, 65.3%, 56.9% and 56.9%, respectively at the same 30% sea water level, which clearly indicate that the efficiency of these treatments were more pronounced in the regenerated plants.

Table (12): Effect of salinity treatments on the grain yield (g)/plant and weight of 1000 grains (g) produced from the regenerated plants of the salt-sensitive Giza 167 wheat cultivar treated with Gamma rays, abscisic acid and putrescine in comparison with those produced by the untreated salt-tolerant Sakha 8 cultivar plants grown in greenhouse under the same saline conditions (%sea water) during 2000-2001 season.

Treatments \ % sea water	0	15	30	45	Mean T	0	15	30	45	Mean T
	Grain yield (g)/plant					Weight of 1000 grains				
Sakha 8 control	1.32	0.88	0.72	0.53	0.86	16.44	13.52	9.49	7.53	11.75
Giza 167 control	1.15	0.66	0.56	0.31	0.67	12.52	9.24	7.09	5.11	8.49
Gamma rays 15 KR	1.97	1.42	1.13	0.07	1.15	21.36	19.24	14.02	0.96	13.90
Gamma rays 20 KR	1.84	1.31	0.14	0.06	0.84	20.61	18.86	2.26	0.94	10.67
Soaking in 2 ppm ABA	1.86	1.33	0.12	0.05	0.84	20.56	18.89	2.25	0.87	10.64
Soaking + spraying with 2 ppm ABA	1.83	1.39	0.11	0.06	0.85	20.54	18.88	2.23	0.83	10.62
Weekly spraying with 2 ppm ABA	1.73	1.42	1.13	0.07	1.09	22.13	19.88	14.12	0.95	14.27
Weekly spraying with 10 µM putrescine	2.01	1.86	1.19	0.08	1.29	22.84	20.72	14.53	0.97	14.77
Mean (S)	1.71	1.28	0.64	0.15		19.63	17.40	8.25	2.27	
New LSD value at		0.05	0.01				0.05	0.01		
Salinity (S)		0.08	0.11				1.10	1.45		
Treatment (T)		0.12	0.15				1.54	2.10		
(S) X (T)		0.23	0.31				3.10	4.10		

Another interesting and important finding can be deduced from such comparison is that, the highly significant increments in the grain yield (g) /plant and the weight of 1000 grains over the respective values of the salt tolerant Sakha 8 control at 30 % sea water level as well as in the Mean T of both yield components, in both parts of this investigation, were only recorded as a result of the weekly spraying with 10 µM putrescine or weekly spraying with 2 ppm ABA as well as the grain irradiation with 15 KR gamma rays. This strongly confirmed the absolute superiority of these treatments in improving

the tolerance to salinity in the treated sensitive Giza 167 wheat plants in the pot experiments and even in their regenerations under the *in vitro* tissue culture experiment so much so that they could approach their optimal productivity which highly significantly exceeded the comparable productivity of both sensitive and the tolerant controls under the same salinity level up to 30 % sea water one, with special referring to the excellence of putrescine in the all cases.

Evidently, the most interesting feature of the obtained data in the present *in vitro* study is that their complete similarity with those obtained in the pot experiment, in the first part of this work (El-Shafey *et al.*, 2003a) as regards, the growth data, chemical compositions and productivity of the treated plants in the first part of this study, with those of untreated calli and regenerated plants regarding growth, chemical compositions and productivity of such regenerated plants in the second part at least, up to 30% sea water level. These findings strongly suggesting that the induced-higher degree of the physiological tolerance to salinity in the regenerated plants under *in vitro* conditions due to the previously applied treatments on the parent plant in the pot experiments, was nearly equal to that of the treated plants in the first part of this work, which enable such regenerated plants to be adapted to the all applied levels of salinity, thus, could successfully complete their life cycle till harvest and even their produced yield was highly significantly exceeded that of both tolerant and sensitive controls under the *in vitro* tissue culture conditions, as did in the pot experiments up to 30% sea water level, nevertheless, without applying any treatments under such *in vitro* conditions. This clearly indicating that the induced tolerance to salinity by the applied treatments, especially the weekly spraying either with 10 µM putrescine or 2 ppm ABA as well as the grain irradiation with 15 KR gamma rays, in the potted plants in the first part was able to be transmissible, with nearly the equal degree, to the regenerated plants under *in vitro* conditions.

Accordingly, it can be concluded that the obtained results in this second part strongly confirmed the previous conclusion drawn from the results of the first one, with special referring to the absolute superiority of the weekly spraying with 10 µM putrescine treatment followed by the weekly spraying with 2 ppm ABA, then, the grain irradiation with 15 KR gamma rays ones, in both parts of the present investigation .

On the other hand, in order to make more assurance and obtain a definite decisive conclusion, the wheat grains produced by the treated salt-sensitive Giza 167 cultivar plants with the most effective mentioned three treatments must be tested in the field, as did under *in vitro* conditions, at least under the saline irrigation with 30% sea water level.

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تأثير أشعة جاما وحمض الابسيسيك والبتروسين على إنتاج نباتات قمح اكثر تحملا للملوحة:

ب- تكوين الكالوس والنباتات وانتاج الحبوب تحت ظروف الملوحة في مزارع الأنسجة

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أجريت تجارب زراعة انسجة بغرض تقييم مدي التحسن في المقاومة للملوحة في صنف القمح الحساس جيزة ١٦٧ نتيجة للمعاملات السابقة في تجارب الأصص في الجزء الأول من البحث وفيها تم اختبار أنسجة الكلس والنموات الخضرية و النباتات الناتجة من أجنة حبوب القمح لجميع المعاملات خلال الموسم الثاني لزراعة الأصص (٢٠٠٠-٢٠٠١) خلال تجارب معملية لزراعة الأنسجة ، انتقلت بعدها النباتات الصغيرة إلى ظروف الزراعة المحمية لاقلمتها واستكمال نموها حتى الحصاد ، باستخدام نفس مستويات الملوحة السابق استخدامها في الجزء الأول من البحث . وقد تطابقت النتائج المتحصل عليها باستخدام زراعة الانسجة مع نتائج تجارب الاصص فيما يتعلق بالتفوق المطلق لمعاملات الرش الأسبوعي اما بتركيز ١٠ ميكرومول بتروسين او ٢ جزء في المليون حمض الابسيسيك و كذلك تشعيع الحبوب قبل الزراعة بجرعة ١٥ كيلوراد أشعة جاما والذي انعكس على تفوق أنسجة الكلس و النباتات الناتجة من حبوب هذه المعاملات الثلاث من حيث وزن الكلس والنمو والتركيب الكيميائي للنباتات النامية والحبوب الناتجة وايضا إنتاجياتها من محصول الحبوب / نبات و كذلك وزن الألف حبة بالجرام والتي فاقت مثيلاتها الناتجة من حبوب المقارنة لصنف سخا ٨ المقارم مسجلة اعلي قيم للزيادات العالية المعنوية أيضا حتى مستوي ٣٠٪ ماء بحر والأهم من ذلك أن هذا التأقلم و هذه المقاومة العالية للملوحة التي تميزت بها النباتات الناتجة من زراعة الانسجة جاءت ذاتية و دون إعادة تطبيق هذه المعاملات مرة ثانية تحت ظروف تجارب زراعة الانسجة ، وهذا يؤكد إمكانية انتقال صفة المقاومة للملوحة من نباتات الأصص المقاومة - بنفس القدر و الدرجة تقريبا - إلي النباتات الناتجة من أجنة حبوبها خلال تجارب زراعة الأنسجة ، والتي مكنتها من التأقلم مع جميع مستويات الملوحة المستخدمة في بيئاتها وبالتالي استكمال دورة حياتها حتى أنتاج محصول الحبوب الذي تفوق تفوقا عالي المعنوية على محصول معاملتي المقارنة سواء للصنف الحساس او المقاوم حتى مستوى ٣٠٪ ماء بحر تحت ظروف تجارب زراعة الأنسجة كما حدث تماما في تجارب زراعة الاصص. ولهذا فان النتائج المتحصل عليها تؤكد علي أن المقاومة للملوحة في النباتات الحساسة لصنف جيزة ١٦٧ وكذلك النباتات الناتجة منها معمليا من خلال تجارب زراعة الأنسجة، يمكن ان تزداد الي اعلي مستوياتها وبالتالي تتحمل الري حتى مستوى ٣٠٪ ماء بحر علاوة على وصولها الى إنتاجيتها المثلى التي تتفوق تفوقا عالي معنويا تحت هذه الظروف على إنتاجية صنف سخا ٨ المقاوم سواء في تجارب الاصص او زراعةالانسجة ، وذلك في حالة رش هذه النباتات الحساسة في تجارب الاصص فقط إسبوعيا اما بالبتروسين بتركيز ١٠ ميكرومول او حمض الابسيسيك بتركيز ٢ جزء في المليون او معاملة حبوبها قبل زراعة الاصص بأشعة جاما بتركيز ١٥ كيلوراد، مع إشارة واجبة الى تفوق معاملة البتروسين الواضح في هذا الشأن.