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EFFECT OF SALINITY AND MUTAGENS ON IN VITRO PERFORMANCE OF POTATOES

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ABSTRACT

This experiment was carried out to study the effectiveness of chemical mutagen sodium azide, SA at different concentrations 0, 0.5, 0.75 and 1 mM. Gamma irradiation at doses of 0, 10, 20, 30 and 40 Gy was also used to test its effect on improving salinity tolerance of the Spunta potato cultivar by shoot tips culture, as well as to produce plants grown under different levels of sea salt 0, 1000, 2000, 4000 and 8000 ppm, aiming to induce and select *in vitro* salt tolerant plant lines. Subcultures were done to the plantlets four times under sea salt concentrations , the obtained results indicated that gradual increase of sea salt levels was negatively correlated with all growth parameters (survival percentage, plant length and number of leaves), survival percentage was 99.72% at the 4th subculture. Exposure to gamma irradiation at low doses (10 and 20 Gy) either alone or combined with sea salt application had stimulative effects on growth parameters, meanwhile higher doses (30 and 40 Gy) adversely affected all the tested parameters. The concentration of 0.75 mM SA improved survival percentage while SA had negative effect on other growth parameters. Concerning RAPD (Random Amplification of Polymorphic DNA) genetic difference between treatments and the control plants were detected. However, the presence of polymorphic bands with three 10-mer primers, enables possible early detection of mutation *in vitro*.

Keywords: In vitro, (solanum tuberosum L.), gamma irradiation, sodium azide (SA), salinity.

INTRODUCTION

Potato *(Solanum tuberosum* L.*)* belongs to the family *Solanaceae*. It is one of the most important vegetable crops grown in Egypt. In many countries potatoes are cultivated in arid and semi- arid regions where shortage or poor water quality are major factors limiting plant growth and yield. Therefore, the use of saline water for crop production is often unavoidable (Heuer and Nadler, 1998). Salinity is an important environmental constraint to crop productivity in arid and semi-arid regions of the world (Foolad, 2004). Potato is moderately salt-sensitive (Paliwal and Yadov, 1980; Naik and Widholm, 1993) , and very sensitive to water stress (Kleinkopf, 1983).the extent of tolerance to salt stress depends on the stress intensity ,the cultivar involved the stage of development of the crop(Levy *et al.*, 1993). Morpurgo, (1991) cited that potato clones grown *in vitro* showed a different response to high NaCl stress condition .These results suggested that *in vitro* system could be used in screening potato germplasm . Bilski *et al.* (1987) reported that yield of potato was reduced to 50% when the conductivities of sulfatic-chlorodic and sulfatic saline soils are 2.16 and 3.38 mmhos cm-^{1} respectively. Naik and Widholm, (1993) found that responses of cultured stem segments and cell suspensions to six levels of sodium chloride (0.0 to 0.25M) differed from those expressed by whole plants in greenhouse, treatments showed a drastic reduction in fresh weight under saline conditions . Shoot culture was considered a reliable approach to evaluate *in vitro* saline stress in potatoes

(Sasikala and Prasad,1994; Martinez and Maestri, 1996; El-aref *et al.* 1998; Khrais *et al.* 1998; Farhatullah and Raziuddin, 2002;). Improvement of potato cultivars by irradiation of *in vitro* cultures offers several advantages over conventional methods of plant breeding which are laborious and time consuming (Das *et al.* 1999). Plant and calli irradiated with gamma rays and treated with chemical mutagenesis through tissue culture have been used to induce variability for tolerance to salinity and resistance to pathotoxins (Lu *et al.* 1993; Thinh, 1993; Zhen, 1993; Ahiabu *et al.* 1995; Sonnino *et al.* 1995; Das *et al.* 1999 and Das *et al.*, 2000). Alternative strategies, especially those based on biotechnology, offer more rapid means of improving existing cultivars. In potato, unfortunately, although salt tolerance is known to be a polygenic trait, the mechanism responsible for salt tolerance has not been studied in detail (Ochatt *et al.* 1999). Tissue culture techniques that make use of somaclonal variation do not require previous knowledge of the genetic basis of resistance, which facilitates *in vitro* selection of potato cells and tissues. However, the acquisition of tolerance to salt has seldom been reported for potato (Sabbah and Tal, 1990). Induction of mutation in potato have been used to induce variability for tolerance to salinity and produced new species (Sonnino *et al.* 1985; Sonnino and Ancora, 1988; Li and Chao, 1994 and El-Fiki, A.A. 1997). *In vitro* culture techniques in combination with mutagenesis can speed up the improvement of potato through breeding.

The main objective of this work was to study the effectiveness SA (Sodium Azide, NaN3) and Gamma irradiation on improving salinity tolerance of the Spunta potato cultivar shoot tips, as well as to produce plants grown under different levels of salinity aiming to induce and select *in vitro* salt tolerant plant lines.

MATERIALS AND METHODS

This study was carried out at the Tissue Culture Laboratory, Vegetable crops Department, Fac. of Agric., Cairo University, in the period between 2003 – 2005 years, aiming to produce potato plantlets more tolerant to salinity by treating the sprouts of tubers with chemical mutagen substance sodium azide (SA), and gamma irradiation treatments.

Plant Source:

Potato tubers *Solanum tuberosum* cv. spunta were obtained from Potato Research Division, Horticulture Research Institute, Agriculture Research Center, Cairo, Egypt.

Preparation of Potato Sprouts:

Potato tubers were washed under running tap water. The surface of tubers were well dried with a towel. Potato tubers were stored in dark condition at room temperature 25±1°C for 10-15 days until sprouting. If the tuber was in the dormancy stage, breaking dormancy was done by soaking the tubers for one hour in 100 mg/l GA3 and stored in dark for 10-15 days at 25±1°C until sprouting (Michael, 1996).

Mutagen Treatments:

Gamma Irradiation:

Sprouting potato tubers were divided into four groups and exposed to 0, 10, 20, 30, and 40 Gry. Irradiation was performed at dose rate of 41.66

rad/sec. Using Co_{60} source from Gamma irradiation chamber unit 4000 at the National Center for Radiation Research and Technology, Naser City, Cairo.

Sodium Azide Treatment:

Sprouts of potato tubers were carefully removed, then washed in running tap water for several time, under aseptic conditions, sprouts were soaked in 20% chlorox (5.25% sodium hypochlorite) for 20 min. A few drops of tween 20 (0.1%) were added to the surface sterilization treatment to enhance spreading. Sprouts were then rinsed 4 times for 5 min. each in sterile distilled water to remove all traces of chlorox. At last sterilized sprouts were soaked with 0, 0.5, 0.75 and 1 mM solution of SA for 3 hours. the solution was prepared by adding the concentrations of SA through micromicron filter (0.45 micron) to distilled water under laminarflow conditions.

Culture of the Excision explants:

Sprouts from irradiated tubers were carefully removed, then washed in running tap water several times, under aseptic conditions sprouts were soaked in 20% chlorox (5.25% sodium hypochlorite) for 20 min. A few drops of tween 20 (0.1%) were added in the surface sterilization treatment to enhance spreading. Sprouts were then rinsed 4 times for 5 min. each in sterile distilled water to remove all traces of chlorox, then shoot tip of the irradiated sprouts and SA treated sprouts were cultured in Murashige and Schoog basal medium (MS) (1962) supplemented with 3% sucrose, 0.2 mg benzyl adenine (BA) /L and 0.1 mg gibberelic acid (GA3)/L, The pH of the prepared medium was adjusted to 5.7 by KOH before addition of agar (7 g/L) . The medium was distributed into the culture jars (325ml) where each jar contained 35 ml of the medium. the culture jars were autoclaved at 121°C for 20 min. then cultured jars were kept in the culture room at a temperature of 22±2°C for 16 h day length. Data recorded after 4 weeks in each jar were surviving % (S), shoot length cm (SL), and number of leave (NL). Survival plantlets then propagated through multiplication phase, this was repeated every 4 weeks until the desired number of *in vitro* plantlets needed for the next experiment was obtained.

Salinity Treatment:

The potato plantlets obtained previously from the multiplication stage were cultured in a medium contains basal MS salts supplement with 3% sucrose, 0.2 mg benzyl adenine (BA) /L and 0.1 mg gibberelic acid (GA_3)/L, to induce salt stress the culture medium was supplemented with 0, 1000, 2000, 4000,and 8000 ppm Sea Salts (from Sigma Company) where every 40g from this salt give 3600 ppm, the pH of the prepared medium was adjusted at 5.7 , then distributed into the culture jars (325 ml) after addition of agar 7g/l , each jar contained 40 ml of the medium, the culture jars were autoclaved at 121°C for 20 min. then cultured jars were kept in the culture room at temperature 22±2°C for 16 h day length. This experiment included 40 treatments, ie. eight mutagenesis (five doses of irradiation and three concentration of SA) and five levels of sea salt. each treatment included 3 replicates, each replicate consisted of 5 jars, and each jar included 5

plantlets. data recorded after 4 weeks in each jar were surviving %(S), shoot length cm(SL) and number of leave (NL). Survival plantlets from this stage which appear salinity tolerance were multiplicited three times at the same medium using the same concentrations, data recorded after 4 weeks in each subculture were surviving % (S), shoot length cm (SL) and number of leaves (NL).

Dna Fingerprints:

DNA fingerprinting was done to the control and the (10 , 20 Gy) gamma irradiated plants and 1 mM SA treatment. DNA was isolated using CTAB method of (Doyle and Doyle, 1987). For RAPD analyses, PCR amplification was performed in 0.01 ml reaction mixture containing 20 ng template DNA, 0.5 unit taq polymerase, 200 µM each of dATP, dCTP, dGTP, dTTP,10 p mol random primer (10 mer) and appropriate amplification buffer. The mixture was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed for 45 cycles at 92°C for 3 min. and then 45 cycles at 92°C for 30 sec., 35°C for 60 sec. and 72°C for 2 min. (for denaturation, annealing and extension respectively). Reaction was finally incubated at 72°C for 10 min. and further 10 min. at 62°C. The amplification products were analyzed by electrophoresis in 2% agarose in TAE buffer, stained with 0.2 µg ml^{-1} ethidium bromide and photographed under UV light. Three random primers were used as illustrated:

OPA-02 5`TGCCGAGC¯G3`
OPA-13 5`CAGCACCCAC3`
OPA-20 5`ACACACGCTG3`

Microtuberization:

Potato plantlets which resulted from the 4^{th} subculture, which showed resistance to different concentrations of sea salt were cultured to produce microtubers. MS solid medium supplemented with 4 mg Kinetin /L, 2 mg calcium-panthocyanat/L ,8% sucrose/L was used, and pH was adjusted at 5.7, incubation was under dark condition at 18°C until microtubers formed, according to Ibrahim, (2004).

Experimental Design:

The experimental design was randomized complete design with three replicates each replicate consisted of 5 jars with five stem cutting per jar. Data were tested by analysis of variance. Duncan's multiple range test was used for the comparisons among the treatments means (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

1- Growth and Development Parameters:

Data present in Tables 1, 2, 3, 4, 5 and 6 show the effect of sea salts levels, sodium azide and gamma irradiation on growth parameters.

1-1 SURVIVAL:

Data presented in Table 1 shows significant differences between subcultures in survival percentage, it was 99.72% for the subculture 4 .

Table 1: Effect of subculture number on growth of potato plantlets, spunta cv.

Treat.	Plant length (cm)	Number of leaves (NO)	Survival (%)
Sub 2	3.825 A	4.732 A	99.17 C
Sub 3	3.733 B	4.733 A	99.33 B
Sub 4	3.685 B	4.650 A	99.72 A

Values within the same column followed by the same capital letter(s) do not significantly differ from each other. According to Duncan's multiple range test at 5%level.

Table 2: Effect of salinity treatment on growth of potato plantlets spunta cv.

Salinity treat.	Plant length (cm)	Number of leaves (NO)	Survival (%)
Control	3.899 C	4.922 C	100.0 A
1000	4.500 A	5.360 A	100.0 A
2000	4.247 B	5.154 B	100.0 A
4000	3.617 D	4.464 D	100.0 A
8000	2.429 E	3.625 E	97.03 B

Values within the same column followed by the same capital letter(s) do not significantly differ from each other. According to Duncan's multiple range test at 5%level.

Table 3: Effect of mutagens treatments (gamma and SA) on growth of potato plantlets, spunta cv.

Mutation treatment		Plant length (cm)	Number of leaves (NO)	Survival (%)
Control		3.642 C	4.753 C	99.29 B
Gamma (Gy)	10	4.478 A	5.242 B	99.73 A
	20	4.558 A	5.431 A	99.51 AB
	30	4.031 B	4.727 CD	99.47 AB
	40	3.498 D	4.584 D	98.93 C
(SA)mM	0.5	3.280 E	4.311 E	99.33 B
	0.75	3.187 E	4.367 E	99.56 AB
	1	3.233 E	4.224 E	99.42 B

Values within the same column followed by the same capital letter(s) do not significantly differ from each other. According to Duncan's multiple range test at 5%level.

The survival percentage for the salinity concentration 8000 ppm was 97.03% whereas it was 100% for the control and the other salinity concentrations (1000, 2000 and 4000 ppm) (Table 2). As for the effect of gamma irradiation rates and SA concentrations on this trait (Table 3) survival percentage for the unirradiated plants (control) was 99.29%,whereas it was 98.93% for irradiated plants (40 Gy). and 99.73% (10Gy).Concerning the interaction between subculture and irradiation data shown in Table 4 indicates that the highest percentage for survival was obtained by the dose of 10 Gy in the three subcultures, it was 100% in subculture 4, however, the lowest percentage of survival was obtained by 40 Gy in the three subcultures. Concerning the SA treatments it was found that there were significant differences between treatments in subculture 2 and 3. The interaction between subculture and salinity concentrations is shown in Table 5. The survival percentage was the

lowest by using the concentration of 8000 ppm in the three subcultures with significant differences between its values (95.83% , 96.67% and 98.58% for subculture 2, 3 and 4, respectively), whereas it was 100% for control and other salinity concentrations (1000, 2000 and 4000 ppm) in three subcultures.

Table 4: Effect of the interaction between mutagens treatments (gamma and SA) and subculture on growth of potato plantlets, spunta cv.

Sub. (NO)	Mutation Treat		Plant length (cm)	Number Of Leaves(NO)	Survival (%)
Sub 2	Co		3.820 d	4.813 b	98.93 ef
	Gamma (Gy)	10	4.547 a	5.247 a	99.47 b-f
		20	4.660 a	5.407 a	99.20 cde
		30	4.160 bc	4.813 b	99.20 cde
		40	3.507 e-h	4.593 b-e	98.67 f
	(SA) mM	0.5	3.373 e-i	4.367 d-g	99.07 def
		0.75	3.260 f-i	4.393 d-g	99.47 b-e
		1	3.273 f-i	4.220 g	99.33 b-e
Mean			3.825 A'	4.732 A'	99.17 C'
Sub 3	Co		3.573 e	4.767 b	99.20 cde
	Gamma (Gy)	10	4.493 a	5.253 a	99.73 abv
		20	4.560 a	5.480 a	99.47 b-e
		30	4.027 cd	4.727 b	99.47 b-e
		40	3.513 e-g	4.607 b-e	98.93 ef
	(SA) mM	0.5	3.240 g-i	4.347 e-g	99.33 b-e
		0.75	3.167 i	4.427 c-g	99.33 b-e
		1	3.287 f-i	4.260 g	99.20 cde
Mean			3.733 B'	4.733 A'	99 33 B'
Sub4	Co		3.533 ef	4.680 bc	99.73 abc
	Gamma (Gy)	10	4.393 ab	5.227 a	100.0 a
		20	4.453 a	5.407 a	99.87 ab
		30	3.907 cd	4.640 bcd	99.73 abc
		40	3.473 e-h	4.553 b-f	99.20 cde
	(SA) mM	0.5	3.227 hi	4.220 g	99.60 a-d
		0.75	3.133 i	4.280 fg	99.87 ab
		1	3.140 i	4.193 g	99.73 abc
Mean			3.658 B'	4.650 A'	99.72 A'

Values within the same column followed by the same small letter(s) do not significantly differ from each other, and values followed by the same capital letter(s) do not significantly differ from each other. According to Duncan's multiple range test at 5%level.

As for the interaction between irradiation, SA and salinity (Table,6) the survival percentage was significantly affected by 8000 ppm in all irradiation and SA treatments. The lowest percentage was found by 8000 ppm with 40 Gy. The obtained results are in harmony with those reported by El Aref *et al.* (1998) and Castillo *et al.* (1997) who mentioned that survival values decreased by increasing the dose of irradiation, And are also in agreement to those of El Barkouki, (2000) who reported that the irradiation and high level of salinity 8000 ppm decreased the percentage of survivals compared to the control.

1-2 Plant Length:

Data calculated in Table 1 show that there was significant differences between subcultures in plant length , The lowest value was obtained in subculture 4. Concerning salinity effect their was significant effect between its concentrations in plant length , on the other hand 1000 ppm caused the highest plant length while 8000 ppm showed the lowest value (Table,2). As for the effect of gamma irradiation rates and SA concentrations on this trait (Table 3) it was found that there were significant differences among treatments except for radiated plants which received 10 and 20 Gy as they gave the highest plant length, on the other hand 40 Gy produced the lowest value among gamma irradiation doses. Concerning the interaction between subculture and irradiation, data in Table 4 show that there were significant differences among treatments in the three subcultures, the highest plant length was recorded due to 10 and 20 Gy in the three subcultures, the lowest value of plant length however was obtained by 40 Gy in the three subcultures .While for the interaction between subculture and salinity concentrations (Table 5) shows that 1000 and 2000 ppm in the three subcultures resulted in the tallest plant length, while 8000 ppm in the three subcultures gave the shortest plant length. As for the interaction between irradiation, SA and salinity (Table,6) plant length was significantly affected by 8000 ppm in all irradiation and SA treatments. The shortest plants were obtained from the interaction between irradiation treatment 40 Gy with 8000 ppm and between 0.75mM SA concentration and 8000 ppm, while The tallest plants were obtained from the interaction between irradiation treatments 10 and 20 Gy with 1000 ppm and 10 Gy with 2000 ppm.

Table 5: Effect of the interaction between salinity treatments and subcultures on growth of potato plantlets, spunta cv.

Sub. (No.)	Salinity Treat (ppm)	Plant length (cm)	Number of leaves (No.)	Survival (%)
Sub 2	Control	3.950 c	4.925 d	100.0 a
	1000	4.621 a	5.346 ab	100.0 a
	2000	4.421 ab	5.183 bc	100.0 a
	4000	3.700 de	4.517 e	100.0 a
	8000	2.433 f	3.688 f	95.83 d
Mean		3.825 A'	4.733 A'	99.17 C'
Sub 3	Control	3.888 cd	4.925 d	100.0 a
	1000	4.463 ab	5.417 a	100.0 a
	2000	4.271 b	5.192 bc	100.0 a
	4000	3.600 e	4.483 e	100.0 a
	8000	2.442 f	3.650 f	96.67 c
Mean		3.733 B'	4.733 A'	99.33 B'
Sub 4	Control	3.858 cd	4.917 d	100.0 a
	1000	4.417 ab	5.317 ab	100.0 a
	2000	4.050 c	5.088 cd	100.0 a
	4000	3.550 e	4.392 e	100.0 a
	8000	2.412 f	3.537 f	98.58 b
Mean		3.658 B'	4.650 A'	99.72 A'

Values within the same column followed by the same small letter(s) do not significantly differ from each other, and values followed by the same capital letter(s) do not significantly differ from each other. According to Duncan's multiple range test at 5%level.

This result is in agreement with those reported by El Barkouki, (2000) who mentained that salinity concentration of 1000 ppm increased plant length while 8000 ppm reduced It. Farhatullah and Raziuddin, (2002) reported that salt stress is related with inhibition of shoot growth, the highest concentration of salts 4000 ppm has a lethal effect on all growth parameters. Li and Chao (1994) reported that only the highest concentrations (1.0% EMS, 0.75 mM SA and 15 kR gamma rays) caused M1 changes in major features such as plant height and weight of aerial parts.

1-3 Numbers of Leaves:

Data presented in Table 1 shows insignificant differences between subcultures in number of leaves. Concerning salinity effect there was a significant effect between its concentrations on number of leaves , the highest value was recorded in 1000 ppm, while the lowest one was by 8000 ppm (Table,2) . Regarding the effect of gamma irradiation rates and SA concentrations on this trait (Table 3) significant differences were obtained among treatments in number of leaves, 20 Gy caused the highest value while 40 Gy caused the lowest value among gamma irradiation doses. otherwise, non-significant differences were detected between SA concentrations. With respect to the interaction between subculture and irradiation, SA concentrations, data in Table 4 show that there were significant differences among treatments in the three subcultures, the highest value was recorded in 10 and 20 Gy in the three subcultures, the lowest value of number of leaves was obtained by SA concentrations in the three subcultures. Concerning the interaction between subculture and salinity in Table 5 number of leaves were the highest in 1000 ppm with the three subcultures, while the lowest number were produced by 8000 ppm in the three subcultures . As for the interaction between irradiation, SA and salinity (Table,6) number of leaves was significantly affected by 8000 ppm in all irradiation and SA treatments. The lowest value were obtained from the interaction between irradiation treatment 40 Gy with 8000 ppm and between SA concentration and 8000 ppm, while The highest value were obtained from the interaction between irradiation treatment 20 Gy with 1000 and 2000 ppm. The obtained results are in harmony with those reported by El Barkouki, (2000) about that salinity concentration 1000 ppm increased number of leaves while 8000 ppm reduced it. Morpurgo, (1988) reported that salinity inhibited development of plants grown *in vitro* on medium containing 154 mM NaCl in all growth parameters (fresh weight, height, nodes/shoot, root fresh weight and number of shoots/flask).

2- DNA Fingerprints:

The application of RAPD analysis was used for the identification of potato cv. Spunta and the detection of differences between the control and the treatment plants with different doses of gamma irradiation and SA concentrations. DNAs were isolated from the control and 10 and 20 Gy, also from 1 mM SA treatments. Three random primers were used as illustrated in Table 7.

Table 6: Effect of the interaction between salinity treatments and mutagens treatments (gamma and SA) on growth of potato plantlets, spunta cv.

Salinity Treat	Mutation Treat		Plant Length (cm)	Number of Leaves (NO)	Survival (%)
Control	Control		3.833 h-k	5.100 f-j	100.0 a
	Gamma (Gy)	10	4.544 c-e	5.533 de	100.0 a
		20	4.622 cd	5.533 de	100.0 a
		30	4.100 f-h	5.200 e-h	100.0 a
		40	3.467 l-p	4.778 i-m	100.0 a
	(SA) mM	0.5	3.511 k-p	4.300 o-q	100.0 a
		0.75	3.356 m-p	4.400 n-p	100.0 a
		1	3.756 h-l	4.533 l-o	100.0 a
Mean			3.899 C'	4.922 C'	100.0 A'
1000	Control		4.733 c	5.433 d-f	100.0 a
	Gamma (Gy)	10	5.122 b	5.900 bc	100.0 a
		20	5.467 a	6.333 a	100.0 a
		30	5.144 ab	5.200 e-h	100.0 a
		40	4.200 fg	5.233 e-h	100.0 a
	(SA) mM	0.5	3.72 i-m	4.933 g-k	100.0 a
		0.75	3.922 g-i	5.111 f-i	100.0 a
		1	3.689 i-n	4.733 j-n	100.0 a
Mean			4.500 A'	5.360 A'	100.0 A'
2000	Control		4.389 d-f	4.789 i-m	100.0 a
	Gamma (Gy)	10	5.278 ab	5.656 cd	100.0 a
		20	5.100 b	6.044 ab	100.0 a
		30	4.744 c	5.300 d-g	100.0 a
		40	4.100 f-h	5.133 f-i	100.0 a
	(SA) mM	0.5	3.333 n-p	4.889 h-l	100.0 a
		0.75	3.644 i-n	4.856 h-l	100.0 a
		1	3.389 l-p	4.567 k-o	100.0 a
Mean			4.247 B'	5.154 B'	100.0 A'
4000	Control		3.233 op	4.444 m-p	100.0 a
	Gamma (Gy)	10	3.978 gi	5.233 e-h	100.0 a
		20	4.278 e-g	5.100 f-j	100.0 a
		30	4.000 g-i	4.344 o-q	100.0 a
		40	3.589 j-o	4.300 o-q	100.0 a
	(SA) mM	0.5	3.422 l-p	4.133 pqr	100.0 a
		0.75	3.178 p	4.133 p-r	100.0 a
		1	3.256 op	4.022 qr	100.0 a
Mean			3.617 D'	4.464 D'	100.0 A'
8000	Control		2.022 r	4.000 qr	96.44 f
	Gamma (Gy)	10	3.467 l-p	3.889 rs	98.67 b
		20	3.322 n-p	4.144 pqr	97.56 cd
		30	2.167 qr	3.589 st	97.33 cd
		40	2.133 qr	3.478 t	94.67 g
	(SA) mM	0.5	2.411 q	3.300 t	96.67 ef
		0.75	1.833 r	3.333 t	97.78 c
		1	2.078 qr	3.267 t	97.11 de
Mean			2.429 E'	3.625 E'	97.03 B'

Values within the same column followed by the same small letter(s) do not significantly differ from each other, and values followed by the same capital letter(s) do not significantly differ from each other. According to Duncan's multiple range test at 5%level.

Table 7: List of 10-mer random primers, their nucleotide sequence and amplification results with the control and the different treatments.

Primer name	Sequence /5→3/	Control	Gamma irrad. Gy		SA 1 mM
			10	30	
		No. of bands			
OPA-02	TGCCGAGCTG	3	4	3	2
OPA-13	CAGCACCCAC	4	4	4	3
OPA-20	ACACACGCTG	2	2	2	2

Primer OPA-02: The results of primer OPA-02 are shown in figure (1-A). It gave a maximum of four amplification products at the molecular sizes which ranged between 400 bp to 1000 bp. The only difference was recorded with 10 Gy gamma irradiation dose and 1mM SA treatment.

Primer OPA-13: The results of primer OPA-13 are shown in figure (1-B). It gave a maximum of four amplification products at the molecular sizes, which ranged between 400 bp to 1517 bp. The only difference was recorded with 1 mM SA treatment.

Primer OPA-20: The results of primer OPA-20: are shown in figure (1-C). It gave a maximum of two amplification products at the molecular sizes which ranged between 500 bp to 1000 bp. It gave DNA product with the control and all treatments. Bands were polymorphic between the control and the treatments. There was no difference between treatments. EL Demerdash, (2000) applied RAPD technique in potato and found genetic differences in potato plantlets of cvs. spunta and saturna irradiated with 40 Gy gamma irradiation. Chemical mutagen cause gene mutation and chromosomal changes, while physical mutagen (gamma irradiation) may cause chromosomal changes rather than gene mutation (Mike and Donnini, 1993; Chahal and Gosal, 2003). It can be noted from these results that there was a genetic difference between treatment and control plants. However, the presence of polymorphic bands with three 10-mer primers, enabling the possible early detection of mutation *in vitro*.

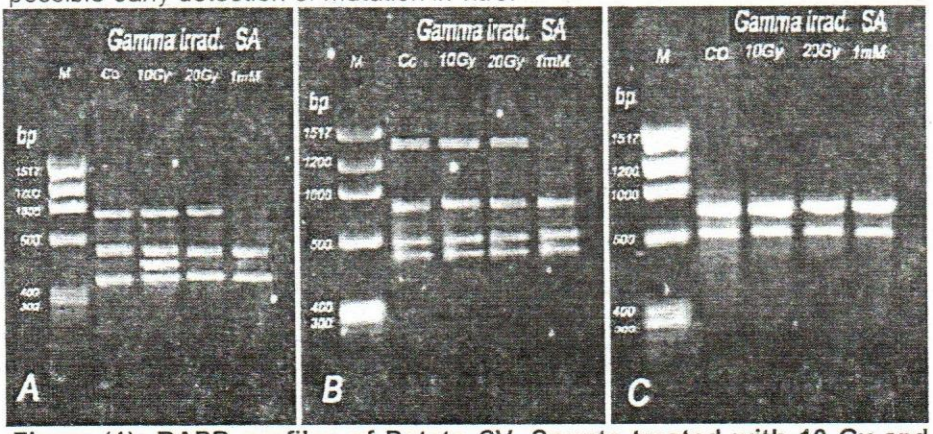

Figure (1): RAPD profiles of Potato CV. Spunta treated with 10 Gy and 20 Gy Gamma irradiation and with 1 mM SA, For three primers: A: OPA-02 primer B: OPA-13 primer, C: OPA-20 primer. CO: control, M: marker.

3- Microtuberization:

It has been noted during plantlets growth on sea salts medium, that remarkable number of microtubers were formed on its shoot, especially at 4000 and 8000 ppm. It was different in shape and color. However microtubers derived from plants which were cultured on microtubrization medium, were collected and kept in dark condition at 4°C to be cultured in greenhouse to produce minitubers for salinity tolerance screening at the field.

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تأثير الملوحة والمواد المطفرة على نمو وتطور البطاطس خلال مزارع الأنسجة

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أجريت هذه التجربة بهدف دراسة تأثير معاملة البطاطس بالمادة المطفرة الكيميائية صوديوم أزايد sodium azide (SA) بتركيزات ٠، ٠,٥، ٠,٧٥، ١ مايكرو مول ، و المعاملة بأشعة جاما بجرعات ٠، ١٠، ٢٠، ٣٠، ٤٠ جراي على تحسين تحمل الملوحة للصنف سبونتا من البطاطس، وذلك عن طريق زراعة القمم النامية. وبهدف إنتاج نباتات قادرة على النمو على مستويات مختلفة من التركيزات التالية من أملاح البحر ٠، ١٠٠٠، ٢٠٠٠، ٤٠٠٠، ٨٠٠٠ ppm . تم إجراء عملية النقل وإعادة الإكثار أربع مرات للنبيتات المعاملة بالمواد المطفرة والمنتخبة من الزراعة الأولى، على نفس التراكيز من الملوحة، حيث أظهرت النتائج أن الزيادة في تركيز الأملاح كانت سلبية التأثير على صفات النمو المدروسة (نسبة الحياة و طول النبات و عدد الأوراق). حيث بلغت نسبة الحياة ٩٩,٧٢% في الإكثار الرابع. كما كان للجرعات المنخفضة من أشعة جاما ١٠ و ٢٠ جراي تأثير محفز على صفات النمو المدروسة، بينما أثرت الجرعات العالية(٣٠ و ٤٠ جراي) بشكل سلبي عليها. أدى التركيز ٠,٧٥ مايكرو مول صوديوم أزايد إلى تحسين نسبة الحياة بينما كان للمعاملة بصوديوم أزايد تأثير سلبي على الصفات الأخرى المدروسة. اظهر استخدام تقنية RAPD وجود اختلافات وراثية بين المعاملات وبين الكنترول، حيث يمكن ظهور الاختلافات الوراثية من أمكانية الكشف المبكر عن حدوث الطفرات .