In vitro Mutation, Selection for Salt-Tolerance and Characterization via RAPD Markers in Banana

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ABSTRACT

The present work was carried out in Pomology Department and Biotechnology Laboratory, Crop Science Department, Alexandria University, during the period from 2000 to 2003.

Small propagules of four banana cultivars namely, Maghrabi, Williams, Grand Nain and Valery were irradiated with the gamma doses 0, 15, 30 or 45 Gy at the rate of 10 Gy min^{-1}, and multiplicated for 10 subcultures. Ten thousands of irradiated shoots from these cultivars were exposed to a salt mixture [3 NaCl +1(3$CaCl_2$ + 1 $MgCl_2$)], which were added to the rooting medium in the rates of 0, 100, 130, 160, 190 mM. The vigorous shoots after 60 days from salinity treatments were selected. The results showed that banana tissues exposed to different doses of gamma irradiation in the range of 15-45 Gy, followed by culturing the plantlets produced in a medium containing additional salts (ranged from 100 to 190 mM) can be considered a good method to identify the most tolerant individuals to salts in banana cultivars. Thus, thirty one shoots from the four banana cultivars were selected as salt-tolerant individuals as well as the shoots grown in salt-free medium as control, recultured in a fresh rooting medium for 60 days, then transferred to a controlled-temperature glasshouse for 3 months of adaptation. Afterwards, these plants were ,daily, irrigated with 20 % sea water for 45 days. Three plants only, which remained vigorous without the appearance of any symptoms of salinity, were considered as salt-tolerant clones and gave abbreviation names : (TW) a salt-tolerant clone of Williams cultivar derived from the irradiation with 30 Gy, (TV) a salt-tolerant clone of Valery cultivar derived from the irradiation with 15 Gy and (TG) a salt-tolerant clone of Grand Nain cultivar derived as variant .

Fourteen primers were used to amplify the DNA of the three salt--tolerant clones and three salt--sensitive plants as control . Fifty two amplification bands were detected and exhibited 100 % polymorphic. Primer OPC-15 revealed that the band (1200 bp) was present in all sensitive-tolerant variants and absent in all salt-tolerant ones. This band may be linked to salt-tolerant in banana. The bands which produced by these primers, were used to construct a similarity matrix, the genetic similarity among these variants ranged from 0.0 to 0.32 and these variants were also classified to four clusters.

The present study indicated that the use of RAPD technique was sensitive and powerful to detect genentic variation at the level of DNA among banana variants. This might be of particular importance in the future, dealing with *in vitro* selection to stress conditions.

INTRODUCTION

Salinity is a widespread problem around the world, especially in arid and semi-arid regions. Each year more and more land becomes non-productive owing to salt accumulation. At least 25% of currently cultivated land throughout

the world suffers from excess salinity (Moxley *et al.*, 1978; Epstien *et al.*, 1980; Nabors, 1990; Bohnert and Jensen, 1996) and all major crop species are intolerant to salt (Fairbairn *et al.*, 2000).

Moderate to high salinity affects about 30 % of the irrigated land in Egypt (Dregne, 1986). Yemen has extensive coastal plains (over 2000 Km), where salinity problems are widespread in this zone, that contains important agricultural areas (FAO / UNCCD / UNDP, 2000).

Recently, Blumwald and Zhang (2001) estimated that 10 millions hectars of agriculturally productive land over worldwide are being lost annually, because of irrigation–induced salinity. Crop production is limited by salinity on 40 % of the world irrigated land. This progressive loss of farmable land is on a collusion course with expanding global production, which over the next 30 years is expected to require an increase in food production of 20 % in developed countries and 60 % in developing nations. The most economic and sustained way to overcome the problem of salt-stress is to develop salt-tolerant varieties (Collins *et al.*, 1990; FAO/IAEA, 1997 a and b; Frommer *et al.*, 1999).

Mutation breeding might be particularly important for sterile Musa species, where there is no sexual reproduction that could generate variation (Krikorian and Cronauer, 1984; De Langhe, 1986). In many vegetatively propagated crops, mutation induction in combination with *in vitro* culture is one of the effective method for plant improvement (Novak, 1991). Vuylsteke *et al.*, (1998) pointed out that banana genetic improvement by conventional hybridization is complex and difficult, yet not impossible. Musa improvement requires a holistic approach to produce better cultivars. Such approach will benefit from these biotechnologies that are clearly valuable in Musa breeding, i. e. tissue culture, diagnostics and molecular markers. Moreover, genetic identification based on genomic DNA analysis is a very important component of modern plant breeding (Antonius-Klemole, 1999; Lagoda *et al.*, 1999).

Plant biotechnology and molecular breeding have already proved their impact in enhancing the productivity of some of the major agricultural crops. They will continue to contribute to the production of plants with novel traits that are otherwise difficult or impossible to develop by conventional breeding (Taji *et al.*, 2002).

In vitro culture in combination with induced mutation can speed up breeding programs, from the generation of variability, through selection, to multiplication of desired genotypes (Maluszynski *et al.*, 1995). Recently, Maluszynski (2001) reported that induced mutation have proven to be most rapid, direct and cheapest approach to develop new plant varieties. Recent advances in molecular characterization of stress related responses and emergence of sensitive molecular analytical tools have reinvigorated research on *in vitro* selection (Jayasankar and Gray, 2003).

The objectives of this study were to (1) Evaluate four banana cultivars for salt-tolerance using *in vitro* culture technique and subsequently,

obtaining salt – tolerant clone(s) by *in vitro* mutation, and (2) Use the RAPD method as a major molecular marker for genetic differentiation among banana variants.

MATERIALS AND METHODS

The present work was carried out in Pomology Department and Biotechnology Laboratory, Crop Science Department, Alexandria University, during the period from 2000 to 2003.

The aim of the present work was to determine the radio-sensitivity of *in vitro* banana culture, which assessed by the survival percentage, number of regenerated shoots and the fresh weight of shoot multiplication in order to select the suitable doses of gamma irradiation to conduct *in vitro* mutation for banana improvement.

Plant materials and culture preparation

Shoot apices of 4 banana cultivars Williams (W), Grand Nain (G), Valery (V) and Maghrabi (M) were used in this study .The basal medium of Murashige and Skoog (1962), supplemented with 80 mg/L adinine sulfate, 0.5 mg/L BAP, 0.5 mg/L NAA, 30 g/L sucrose and 5 g/L agar was used for the initial culture and the multiplication stage (Al-Murish, 2003). The pH was adjusted to 5.8 before autoclaving. The medium was dispensed into screwtopped glass jars (350 ml), each receiving 60 ml. Cultures were incubated at 25 ± 2° C under 16 h illumination (2000 lux, daylight fluorescent tubes) .

Effect of gamma irradiation on *in vitro* shoot culture

Small propagules of the cultivar 'Williams' were transferred to autoclavable plastic tubes containing 20 ml of half–strength MS salts. The propagules were irradiated in a gamma cell with a 60Cobalt source at National Center of Radiation Research and Technology, Nasr City, Cairo, Egypt, with the doses of 0, 15, 30, 45, 60 or 75 Gy at a dose rate of 10 Gy min^{-1}. The irradiated propagules were removed from the tubes and recultured on a fresh proliferation medium.

After forty days incubation, the impact of the irradiation was assessed by determination the survival percentage of cultures, the number of multiplied shoots and the increase of fresh weight of cultures for each treatment.

In vitro selection

Small propagules of 4 banana cultuivars, namely, Maghrabi, Williams, Grand Nain and Valery were irradiated in a gamma cell with a 60Cobalt source with the doses of 0, 15, 30 and 45 Gy at a dose rate of 10 Gy min-1. The cultivars were subcultured ten times (multiplied for 10 subcultures) before *in vitro* selection for salt–tolerance.

Individual shoots from the irradiated cultures (ten thousands shoots multiplied from the four cultivars) were cultured on the rooting medium (Al-Murish, 2003) supplemented with different concentration of a salt mixture [3 NaCl : 1 (3 CaCl2 + 1 MgCl2) Ibrahim and El-kobbia (1986)]. The concentration of the salt mixture were 0, 100, 130, 160 or 190 mM. After sixty days of incubation, the vigorous shoots were selected and transferred to fresh medium free from salt mixture. The plantlets were transferred to greenhouse for acclimatization and selection for salt–tolerance, again, at whole plant system.

The selected and unselected (control) plantlets were planted in plastic pots (300ml) containing a peat moss: perlite (3:1,v/v) and kept at a controlled–temperature glasshouse. These plants were daily fertigated with Kristalon compound (19N: 19P :19K) at gradual rate 1 to 3 g l^{-1} following to their age .

Three months later, the selected and unselected (control) plants were daily irrigated with 20 % sea water (sea water = 530 mM NaCl) , with continuous fertilizer supplement, at rate of 50 ml pot-1 for 45 days. Three plants only were remained vigorous without appearing of any symptoms of salinity effects.

RAPD markers

Plant materials

Polymerase chain reaction (PCR) analyses were carried out using the genomic DNA from three variants tolerance to salinity and three variants sensitive to salinity which obtained from *in vitro* selection.

DNA extraction

Frozen young leaves (1g) were ground to a powder in a mortar with liquid nitrogen. The powder was poured into tubes, containing 9.0 ml of warm (65 °C) CTAB extraction buffer . The tubes were incubated at 65 °C for 60 - 90 min. Additional 4.5 ml of chloroform / octanol (24:1) was used and tubes were rocked to mix for 10 min. and centrifuged for 10 min. at 3200 rpm. The supernatants were pipetted into a new tubes and 6 ml of isopropanol was added. After 60 min, the tubes were centrifuged for 10 min. and the pellets obtained were put in sterile Eppendrof tubes, containing 400 µl of TE buffer of a pH 8.0 (10 mM Tris- HCl, pH 8.0 + 1.0 mM EDTA, pH 8.0) (Sagahi-Maroof *et al.*, 1984). The DNA's from genotypes were, thus, extracted and stored at –20 °C until use.

PCR amplification

Forurteen primers (Table 4), obtained from Pharmacia Biotech. (Amersham Pharmacia Biotech UK Limited, England HP79 NA), were tested to amplify the templated DNA of both salt–tolerant and sensitive variants.

Amplification reaction volumes were 25 µl, each containing 1X PCR buffer with $MgCl_2$ (50 mM KCl, 10 mM Tris-HCl, pH = 9.0), 2 mM $MgCl_2$ and 1% Trition X-100), 200 µM each dATP, dCTP, dGTP and dTTP, 50 PM primer, 50 ng template DNA and 1.5 µl of taq polymerase. Reaction mixtures were

exposed to the following conditions : 94 °C for 3 min, followed by 45 cycles of 1 min. at 94 °C, 1min. at 63 °C, 2min. at 72 °C, and a final 7 min. extension at 72 °C.

Amplification products were vizualized with DNA marker on 1.6 % agarose gel with 1X TBE buffer and detected by staining with an ethidium bromide solution for 30 min. Gels were, then, destained in deionized water for 10 min. and photographed on Polarid films under UV light.

Data handling and cluster analysis

Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. If a product was present in a genotype, it was designated as "1", if absent it was designated as "0" after excluding the unreproducible bands. Pairwise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients, according to Jaccard (1980). The similarity coefficient were, then, used to construct dendrograms, using the unweighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical, and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program Rohlf, 1993).

Statistical analysis

In vitro evaluation for salt–tolerance the study was conducted in a 2–factor experiment (cultivar and salt concentration), in a completely randomized design (CRD) with 10 repetitions (each treatment = 10 rep. x 5 explants). Effect of gamma irradiation on *in vitro* shoot culture was performed in a completely randomized design (CRD) with 5 repetitions (each treatment = 5 rep. x 5 explants).

The analysis of variance of the data as well as the mean comparisons by least significant difference test (LSD) in all the above studies were performed using Genstat program 5 Release 3.2 (1995). RAPD data were analyzed with *Totl*Lab. Software V. 1.11.

RESULTS AND DISCUSSION

Effect of gamma irradiation on *in vitro* banana culture

For an effective use of mutation induction in plant breeding programs, the basic requirement is the analysis of radio-sensitivity of the explant material (Walther and Sauer, 1986). Concerning banana, after irradiation of excised shoot-tips, *Musa* cultivars exhibited significant differences in radio-sensitivity and post-irradiation recovery, assessed as fresh weight increase (Novak *et al.*, 1990). Recently, Predieri (2001) reported that one of the first steps in mutagenic treatment is the estimation of the most appropriate dose to apply.

Statistical analysis for the number of shoots and the fresh weight of multiplied shoots derived from the irradiated explants revealed highly significant differences among irradiation doses (Table 1).

Forty days after irradiation of small propagules of banana cv. Williams cultured *in vitro*, no reduction in the survival percentage was observed with the irradiation doses up to 45 Gy, then a sharp decrease was marked by the dose 60 Gy. The higher dose (75 Gy) was completely lethal (death 100 %) (Table 2).

Data in Table (2) indicated that clear decreasing in number and fresh weight of proliferated shoots per explant were occurred with the increasing of irradiation doses. Previously, Similar results were reported by several investigators (Novak *et al.*, 1990; Kulkarni *et al.*, 1997). Novak *et al.*, (1990) reported that *Musa* clones exhibited differences in radio-sensitivity and post-radiation recovery. Ploidy level and hybrid constitutions by genomes A (*acuminata*) and B (*balbisiana*). They also reported that considerable phenotypic variation was observed among plants regenerated from *in vitro* shoot-tips after mutagenic treatment. Kulkarni *et al.*, (1997) reported that the effect of gamma irradiation on multiple shoots was characterized by a drop in multiplication ratios, 70 Gy and more being totally lethal.

Several studies have been ,also, conducted on the radio-sensitivity of *in vitro* cultures of fruits, such as *Prunus avium* (Walther and Sauer, 1985), Kiwifruits (Shen *et al.*, 1990), grapevine (Lima da Silva and Doazan, 1995; Charbaji and Nabulsi, 1999) and *Prunus salicina* (Predieri and Gatti, 2000). Previously, Laneri *et al.*, (1990), working with *Gerbera jamesonii*, stated that in a mutation breeding experiment, the dose chosen for the main experiment should result in the highest survival of irradiated explants and that a low inhibition of the rate of production of new shoots gives the highest efficiency in recovering useful mutants.

Based on these studies, the obtained results in the present investigation suggested that the doses of 15 to 45 Gy seem to be the more suitable to induce mutation for banana improvement.

In vitro shoot-tip culture has been proposed as a system for mutation induction in banana and plantains (Novak *et al.*, 1987). This method offers several advantages for mutation induction in vegetatively-propagated plants (Novak and Micke, 1988): (i) vegetative shoot apices can be ,rapidly, propagated *in vitro* before mutagenic treatment; (ii) the treatment applied to an apical meristem will give rise to a mericlinal chimera. Periclinal and / or homohistont structures can be obtained by repeated culture and formation of axillary and /or adventitious meristems derived from the treated apex, and (iii) intrasomatic competition may be controlled by modifying *in vitro* conditions (e. g. medium composition) resulting in changed survival rates of mutated cell progenies.

In vitro mutation and selection for salt-tolerance in banana.

The conventional breeding of bananas is extremely difficult owing to it's vegetative mode of propagation, problems in crossing and obtaining viable seeds, inherent polyploidy and long life cycle. To overcome these barriers, mutation breeding can be employed as a promising technique and the diversification of *Musa* species can be achieved. Induced mutations change only one or a few specific traits of an elite cultivar without undesired additional variations (Predieri, 2001). He concluded that the most suitable method may be the mutation treatment and propagation of *in vitro* axillary shoots without passage through undifferentiated growth, and can contribute to fruit improvements without upsetting neither the requirements of the fruit industry nor the consumers.

Through *in vitro* selection, mutation with useful agronomic trait, .e.g. salt or drought tolerance or disease resistance, can be isolated in a short duration (Jain, 2001).

The number of vigorous shoots of four banana cultivars showed a marked differences in their *in vitro* salinity tolerance (Table 3). It is clear from the table that the number of vigorous shoots decreased rapidly with increasing salinity.

The highest number of vigorous shoots were obtained with the cultivars "Maghrabi" (3) and Grand Nain (3) on the rooting medium supplemented with 100 mM selective agent of salinity when the propagules exposed to 15 and 30 Gy, respectively (Table 3).

The effect of gamma–rays on the number of vigorous shoots is shown in Table (3). It is clear that the number of vigorous shoots was affected by gamma–ray doses. The number was decreased with increasing irradiation doses. The numbers were 12, 10 and 9 vigorous shoots at 15, 30 and 45 Gy, respectively.

The thirty one shoots from the four banana cultivar (Table 3) which selected as salt-tolerant individuals and shoots grew in salt-free medium as control were recultured in a fresh rooting medium for 60 days then transferred to a controlled–temperature glasshouse for 3 months acclimatization. These plants were daily irrigated with 20 % sea water for 45 days. Three plants only, which remained vigorous without appearing of any symptoms of salinity were considered as salt-tolerant clones and gave abbreviation names : (TW) a salt-tolerant clone of "Williams" cultivar derived from the irradiation with 30 Gy, (TV) a salt-tolerant clone of Valery cultivar derived from the irradiation with 15 Gy and (TG) a salt-tolerant clone of Grand Nain cultivar derived as variant .

In vitro shoot culture could be a better system for testing and selecting for salt–tolerance Martinez *et al.,* (1996). Previously, Chandler *et al.,* (1988) reported that one method of overcoming the loss of regenerative ability in selected cell lines is to carry out the selection using differentiated cultures incubated on medium containing NaCl. In relation to callus culture, this method has the advantage that given the stability of shoots multiplied by apices or axillary buds (Hu and Wang, 1983).

In vitro axillary bud cultures and crude sea salt have been used to identify and / or develop salt–tolerant of potato varieties by Potluri and Prasad (1993). They ,clearly, showed the usefulness of this system to study the screening and physiology of salt–tolerant varieties, as it is a controlled system without the problems of regeneration after exposure to stress. Also, Zhang and Donnelly (1995) proposed *in vitro* procedure, using stem segments, as a quicker and reliable method for salt–tolerance screening of potato cultivars.

FAO/IAEA (1997 a and b) reported that plant biotechnology in combination with mutation induction and conventional breeding might open new frontiers for obtaining rice varieties of salt-tolerants. The application of mutation techniques in breeding has increased constantly over the past years. These techniques must be rapid to keep pace with the large amount of breeding materials generated after mutagenesis. Screening under field conditions is difficult due to several factors such as stress heterogeneity, the presence of salt related stress, and the significant influence of environmental factors such as temperature, relative humidity and solar radiation.

Genetic modification of crop plants to improve their salt–tolerance is a possible way of increasing production, especially for regions of the world where arable lands must be extended to marginal area, and sometimes irrigated with saline water (Dorion *et al.*, 1999).

In conclusion, in the genus *Musa*, conventional breeding is limited by the high sterility and polyploidy. Mutation induction coupled with shoot tip culture are an effective system for the improvement of a modern cultivar. Moreover, *in vitro* propagated plants are ,increasingly, becoming the plant material of choice because of disease control, uniformity and the possible multiplication of a valuable genotype (FAO / IAEA ,2002).

RAPD markers for salt – tolerance in banana

Screening for polymorphic primers

DNAs were extracted from both the three *in vitro* salt-tolerant variants (TW, TV, TG) and three salt–sensitive ones (SW, SV, SG) of three banana cultivars; namely, Williams, Valery, and Grand Nain. Fourteen random 10-mer primers (Table 4) were used to amplify the genome of both groups of variants. A total of 52 DNA fragments (bands) were generated by those primers, and all of these amplified fragments were polymorphic (100 %). The number of bands, amplified per primer ranged from 0 (primers A1, A2, A3, A4) to 10 (primer OPC–15) with a mean value of 3.71 bands per primer (Table 4). The size of fragments ranged from 600 to 1600 bp. However, figure (1) shows the amplification profiles, generated by primer OPC-15 across the banana variants. All of the ten scorable bands were polymorphic across the banana variants. The DNA fragment with molecular weight of 1200 bp (Figure 1) is presented in the three *in vitro* salt- sensitive variants of the three tested banana cultivars, but absent in the three *in vitro* salt-tolerant ones. Similar results were obtained by Ochatt *et*

al., (1999). Their results showed a loss of a band in three salt tolerant clones of potato compared to control.

Other DNA fragment (600 bp) were obtained in the present study with the primer OPU – 06 in the salt –tolerance variant of Grand Nain cultivar may be also associated with the tolerance of salinity in banana, (Figure 2). Moreover, other bands were scored either in the salt –tolerant or salt- sensitive variants in this work. Previously, a high level of polymorphism for RAPD method was reported by Howell *et al.*, (1994), who used a random primers to amplify DNA segments and 116 amplification products were obtained. Genetic variation, detected with RAPD markers among *Musa* germplasm, have also been reported by several investigators (Grajal-Martin *et al.*, 1998; Vuylsteke *et al.*, 1998).

Recently, DNA polymorphism among banana somaclones, using RAPD markers, have been reported (Vidal and De Garcia, 2000). Their results indicated that highly reproducible RAPD patterns can be obtained when applying the DNA extraction protocol to banana leaves.

Williams *et al.*, (1990) reported that polymorphism among individuals could arise through nucleotide change that prevented amplification by introducing either a mismatch at one priming site, detection of a priming site, insertions that rendered priming sites to distant to support amplification and insertions or deletions that changed the size of the amplified product.

The PCR technique proved to be a powerful tool for the identification of polymorphism in lemon (Deng *et al.*, 1995), peach (Hashim *et al.*, 1997), olive (Besnard *et al.*, 2001), mango (Kumar *et al.*, 2001) and *pyrus* (Schiliro *et al.*, 2001).

Cluster analysis

One of the goals of the present study was to investigate the efficiency of RAPD markers in determining, accurately, the genetic relationship between the salt–tolerant variants and the salt–sensitive ones in three banana cultivars.

The RAPD markers, produced by fourteen primers, were used to construct a similarity matrix (Table 5). Simple matching coefficient, ranging from 0.00 to 0.32, suggested a broad genetic base for these variants of banana. Table (5) indicates the genetic similarity estimates of the 15 pairwise comparisons among the variants, based on the 52 polymorphic bands.

However, figure (3) represents the clustring of the salt–tolerant variants (TW, TV, TG) and salt–sensitive ones (SW, SV, SG) generated by UPGMA analysis. Four culsters could be observed. The first cluster included only the TW variant, while the second one include the SW variant. The third culster include the TV, TG and SV variants and the last one included only SG variant. It can be seen from this figure that the shortest genetic distances (the highest similarity value) was found between the two salt-tolerant variant TV and TG, whereas the

highest distance (the low similarity value) was observed in the TW variants, which was separated in an individual cluster.

These results indicated that RAPD technique could be successfully applied to species with very large genomes like banana to obtain a proper characterization of genetic relationship. Howell *et al.*, (1994) stated that RAPD markers were more easily handled and thus, became more desirable to estimate genetic relationship among *Musa* germplasms. Comparison of DNA marker and pedigree–based methods of genetic analysis of plantain and banana clones have been reported (Tenkouano *et al.*, 1999). Their results indicated that there was no association between marker–based similarity and pedigree relationships. They suggested that pedigree–based analysis may prove useful for the selection of prospective parental combinations in *Musa* breeding. While DNA markers may provide a more accurate description of genetic relatedness.

Recently, molecular markers are considered to provide a better estimate of genetic diversity as they are unaffected by environmental factors which may affect the phenotype. Moreover, these markers provide a vast number of descriptors that can be used in addition to morphological data where these are unable to distinguish varieties (Jackson *et al.*, 2000).

In conclusion, these results must be regarded as preliminary because of the small sample size analyzed and the low number of used primers, and the low number of generated RAPD bands. Nevertheless, they are encouraging. Moreover, it gives information on the level of genetic polymorphism existing among these variants and brings new perspectives for the use of such markers in a breeding program of salt–tolerance for banana.

The present study indicated that the use of RAPD technique was sensitive and powerful to detect genentic variation at the level of DNA among banana somaclones. This might be of particular importance in the future, dealing with *in vitro* selection to stress conditions.

Table 1. Analysis of variance for number of shoots and the fresh weight of multiplied shoots of banana cv.'Williams' shoot tips cultured *in vitro* for 40 days after gamma irradiation treatments .

Source of variance	d.f.	MS	
		No. shoots explant $^{-1}$ [(a)]	Shoots fresh weight (g)
Irradiation doses	5	16.657 **	554.36 **
Error	54	0.2693	4.1240

(a) Data were transformed to square root transformation.
** Significant at 0.01 level of probability .

Table 2 . Effect of gamma irradiation, 40 days after the treatment, on *in vitro* banana shoot culture cv. 'Williams' : % survival, number of shoots and the fresh weight of multiplied shoots .

Gamma irradiation doses	% survival of cultures	No. of shoots explant $^{-1}$ [(a)]	Shoots fresh weight
0	100	18.90 A	18.88 A
15	100	17.70 B	16.96 B
30	100	14.60 C	13.55 C
45	100	10.90 D	9.88 D
60	60	5.90 E	3.77 E
75	0	0 F	0 F

(a) Data were transformed to square root transformation.
Means within a column followed by the same letter are not ,significantly, different at the 0.05 level of probability.

Table 3. Effect of irradiation doses on the number of vigorous shoots of four banana cultivars, after 60 days from culturing in media containing different salt concentrations.*

Irradiation doses (Gy)	0				15				30				45				Total
Cultivars / Salt Concen. mM	M	W	G	V	M	W	G	V	M	W	G	V	M	W	G	V	
100	0	0	0	0	3	2	0	2	2	0	3	0	0	1	2	2	17
130	0	0	0	0	0	2	0	0	0	0	2	0	0	0	0	1	5
160	0	0	0	0	2	0	0	0	1	2	0	0	0	1	0	0	6
190	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	3
Total	0	0	0	0	5	4	0	3	3	2	5	0	0	2	4	3	31

* Number of cultured shoots = 10 000
Selective agent of salinity = [3 NaCl : 1 (3 $CaCl_2$ + 1 $MgCl_2$)].

Table 4: Number of amplification and polymorphic products using 14 primers, in banana salt–tolerant clones (TW, TV, TG) and salt–sensitive clones (SW, SV, SG).

Primers	Sequence '5 – '3	No. of Amplification bands(a)	No. of polymorphic bands (b)	Polymorphism % (b / a)
OPC – 15	GACGGATCAG	10	10	100
OPJ – 10	AAGCCCGAGG	8	8	100
OPZ – 03	CAGCACCGCA	6	6	100
OPC – 04	CCGCATCTAC	5	5	100
OPU – 06	ACCTTTGCGG	5	5	100
OPA – 02	TGCCGAGCTG	5	5	100
OPH – 13	GACGCCACAC	4	4	100
A6	CGATCGATGC	4	4	100
OPJ – 04	CCGAACACGG	3	3	100
OPA – 06	GGTCCCTGAC	2	2	100
A1	GTTGCGATCC	0	0	0
A2	TCGCCAGCGA	0	0	0
A3	GGAAGCTTCG	0	0	0
A4	GCGGTACCCG	0	0	0

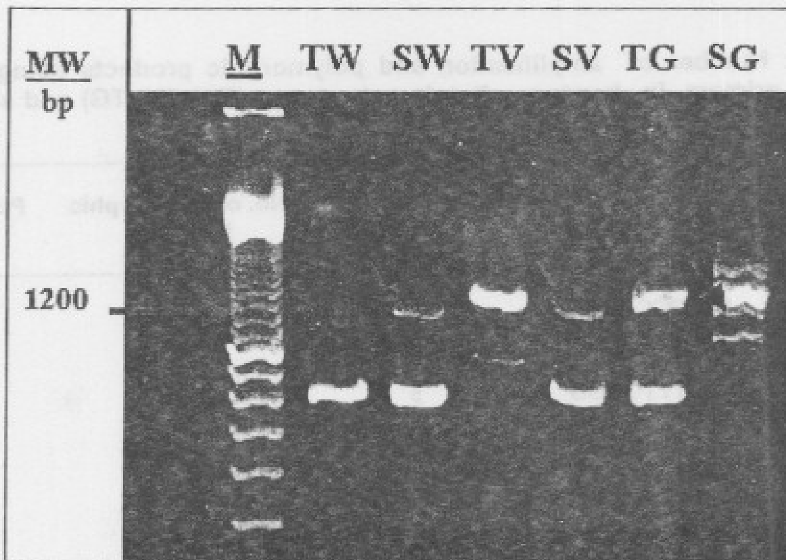

Figure 1. RAPD profiles, with primer OPC–15, of banana salt–tolerant clones (TW, TV, TG) and salt–sensitive plants (SW, SV, SG). The band 1200 bp is absent in all banana salt–tolerant clones.

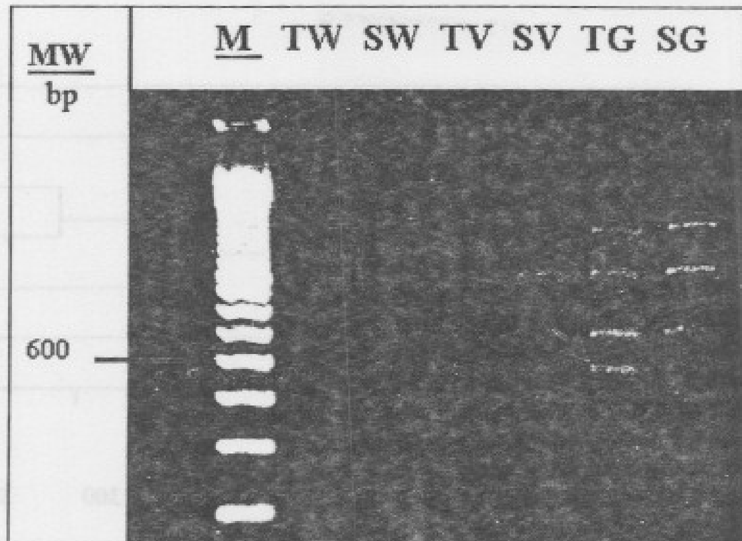

Figure 2. RAPD profiles, with primer OPU – 06, of banana salt–tolerant clones (TW, TV, TG) and salt–sensitive plants (SW, SV, SG). The band 600 bp is present in salt–tolerance clone (TG).

Table 5. Similarity matrix for banana salt–tolerant variants (TW,TV,TG) as compared with salt–sensitive varinants (SW,SV,SG), constructed by Jaccard's similarity coefficients and RAPD data.

	TW	SW	TV	SV	TG	SG
TW	1.00					
SW	0.16	1.00				
TV	0.04	0.22	1.00			
SV	0.00	0.21	0.25	1.00		
TG	0.05	0.25	0.32	0.27	1.00	
SG	0.00	0.04	0.06	0.13	0.18	1.00

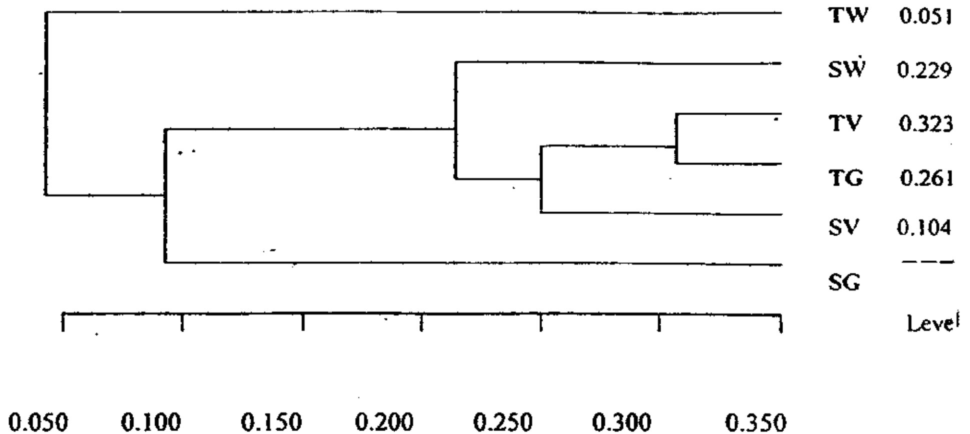

Figure 3 . Dendrogram of genetic distance, constructed using RAPD data and UPGMA method of clustring, shows DNA similarity between salt – tolerant variants (TW,TV,TG) and salt – sensitive ones (SW,SV,SG) of three banana cutivars.

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الملخص العربي

التطفير والانتخاب *In vitro* لمقاومة الملوحة باستخدام تقنية الـــ RAPD Markers في الموز

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أجريــت هــذه الدراســة خلال الفترة من عام ٢٠٠٠ حتى عام ٢٠٠٣ . تم تعريض نموات خضرية لأربعة أصناف من الموز هي المغربي وويليامز وجراندناين وفاليري لجرعات مختلفة من أشعة جاما (صفر ، ١٥ ، ٣٠ ، ٤٥ جــراي بمعــدل ١٠ جراي / دقيقة) وتم إكثارها لمدة عشرة نقلات Subcultures ومن خلالها تم الحصول على عشرة آلاف نمو خضري مشعع . تم زراعة تلك النموات على بيئة التجذير التي تحتوي علــى تركــيزات صفر ، ١٠٠ ، ١٣٠ ، ١٦٠ ، ١٩٠ ملليمول من مخلوط الملح [٣ كلوريد صوديوم : ١ (٣ كلوريد كالسيوم + ١ كلوريد ماغنيسيوم)] . تم انتخاب ٣١ من النباتات الناتجة والتي ظلت قوية النمو بعد فترة ٦٠ يــوم من تعريضها للملوحة . ونقلت هذه النباتات إلىالصوبة بعد إعادة زراعتها على بيئة تجذير خالية من الملوحــة لتكويــن مجموع جذري قوي ثم تم عمل Screening للنباتات المنتخبة في عمر ثلاثة شهور بريها بملوحــة ٢٠ % مــن ماء البحر لمدة ٤٥ يوم وقد تم الحصول على ثلاث سلالات متحملة للملوحة وهي سلالة (TW) مــن الصــنف Williams ناتجة من التشعيع بجرعة ٣٠ جراي وسلالة (TV) من الصنف Valery ناتجة من التشعيع بجرعة ١٥ جراي وسلالة (TG) من الصنف Grand Nain ناتجة كـ Variant .

تــم اســتخدام ١٤ من البادئات Primers في الكشف عن دلائل Markers مرتبطة بصفة تحمل الملوحة في ثلاث سلالات متحملة للملوحة وكذلك تحديد درجة التماثل الوراثي لها مقارنة بثلاث سلالات أخرى حساســة للملوحة (كنترول) . تم الحصول على ٥٢ حزمة (Band) وكانت ١٠٠% Polymorphic وأظهر الــبادئ OPC-15 وجود حزمة (1200 bp) في السلالات الحساسة وغائبة في السلالات المتحملة للملوحة وتــم عمل Similarity matrix من نتائج البادئات Primers المستخدمة وتراوحت قيمة التماثل الوراثي بين تلــك الســلالات ما بين صفر إلى ٠,٣٢ وبناء على تحليل التجمع Cluster الناتج من تلك البيانات فقد قسمت السلالات المدروسة إلى أربع مجاميع Four clusters .

يتضــح من هذه الدراسة أن استخدام تقنية الـــ RAPD markers كانت طريقة حساسة وفعالة في الكشــف عــن الاختلافات الوراثية على مستوى الحمض النووي DNA فيما بين الاختلافات الوراثية الجسمية للمــوز وهــذه التقنية يمكن أن يكون لها أهمية كبيرة في المستقبل إذا ما ارتبطت بعملية الانتخاب ضد ظروف الإجهاد باستخدام تقنية زراعة الأنسجة (*In vitro*) .