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INDUCING AND SCREENING FOR SALT-TOLERANT BANANA *In vitro*

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ABSTRACT

Gamma irradiation was utilized for inducing mutations in banana *in vitro*. Tissue culture techniques were used for selecting salt-tolerant mutants. Immediately after a subculture, explants of `Grand Nain', `Gros Michel' and `Williams' bananas were exposed to 40 or 60 Gy gamma irradiation dose using Mega-Gamma-1 type J6600 cobalt-60 irradiator. Explants derived from gamma-irradiated tissues were tested for radio sensitivity. Radiation sensitivity was assessed as percentage of survival, number of the proliferated shoots, shoot length and wet and dry weights of the proliferated shoots. The studied banana genotypes exhibited variation in radio sensitivity. Mutagenic treatments caused morphological variants. The most obvious one is albino. For screening of salt-tolerance after gamma irradiation, each explant was considered as a mother for an individual line. *In vitro* raised shoots of each line were tested for salt-tolerance in media specifically formulated to induce salt stress with selected levels of NaCl; 0.25, 0.75 or 1.25%. Among gamma irradiated tested lines of `Williams', one mutant line showed enhancement of growth traits compared with the mother tissues under salt stress. This line was obtained by 40 Gy gamma irradiation. Survival rate of the `Williams' salt-tolerant mutant line maintained stable with increasing concentration of NaCl in the media up to 0.75%. Fifteen percent of the `Williams' salt–tolerant mutant line explants survived NaCl at 1.25%. NaCl at 0.25% had no significant effect on shoot number of

the salt mutant line. However, a sudden decrease in number of shoots per explant at 0.75% NaCl was observed. NaCl at 0.25% had no effect on shoot length of `Williams' salt-tolerant mutant line, however NaCl at 0.75% increased the shoot length. No reduction in shoot wet weight in the `Williams' salt-tolerant mutant line was noticed in response to 0.25% NaCl. The reduction in shoot wet weight of the mother `Williams' was more than that of the salt-tolerant mutant line grown in media containing 0.75% NaCl. Changes in protein and isozyme synthesis were investigated to provide information on the mechanism of the salt-tolerance of `Williams' mutant line. The salt-tolerant mutant line showed absense of one protein of 146.5 KD MW that characterizes the mother `Williams' and induced three other proteins of 43.2, 70.0 and 129.3 KD MW. The electrophoretic patterns of esterase, peroxidase or malate dehydrogenase isozymes showed differences between the salt-tolerant mutant line and the mother `Williams'.

Key words: *banana, gamma irradiation, inducing mutations, in vitro, salt-tolerance, tissue culture.*

1. INTRODUCTION

Recent developments emphasise the potential of using tissue culture as a tool for genetic improvement (Piepho and van Eeuwijk 2001). Utilizing tissue culture techniques for screening and developing salt-tolerant varieties has been increasing rapidly (Tal 1983). The genetic nature of *Musa* is suitable for the application of *in vitro* inducing mutations as a method of genetic improvement (Novak *et al.* 1987). Mutation as a sudden change in the nucleotide sequence of DNA, might be created by the application of gamma irradiation; one of the physical mutagens (Rayns 1993). *In vitro* inducing mutations by gamma irradiation allows the quick and efficient screening of a large number of mutant plants (Domingues *et al.* 1994). Developing techniques for selecting variants under salt stress by monitoring the physiology of shoots *in vitro* instead of testing whole plants *in vivo* will provide a relatively simple method of selection (ElObeidy *et al.* 2002).

The objectives of this work was utilizing gamma irradiation as a way of inducing mutations *in vitro* and employing tissue culture techniques for selection of salt-tolerant mutants.

2. MATERIALS AND METHODS

This study was carried out at the Faculty of Agriculture, Cairo University, during 1998 and 1999 on three banana cultivars; 'Grand Nain', 'Gros Michel' and 'Williams'. Shoot tip cultures were established on a modified MS medium supplemented with sucrose and 22 μM BA. The medium pH was adjusted to 5.7 prior to the addition of 0.5% agar. Cultures were maintained at 27°C under continuous photosynthetic photon flux provided by fluorescent lamps. The proliferated shoots were subcultured onto fresh medium every six weeks.

Immediately after a subculture, explants were exposed to 40 or 60 Gy gamma irradiation dose using Egypt's Mega-Gamma-1 type J6600 cobalt-60 irradiator at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt.

Explants derived from gamma-irradiated tissues were tested for radio sensitivity. Radiation sensitivity was assessed by percentage of survival, number of the proliferated shoots, shoot length and wet and dry weights of the proliferated shoots. Tests were carried out forty days after irradiation.

For screening for salt-tolerance, a subculture was carried out six weeks after exposure to gamma irradiation. Each explant was considered as a mother for an individual line. Subculture was carried out four times to establish a population from each explant.

In vitro raised shoots of each line were used as experimental materials for screening for salt tolerant mutants. Media specifically formulated to induce salt stress were supplemented with 0.25, 0.75 or 1.25% NaCl. Cultures were allowed to grow for 8 weeks at selected growth conditions, then tests were carried out and data were recorded.

Sodium dodecyle sulphate (SDS) polyacrilamide gel electrophoresis was conducted according to the method described by Laemmli (1970) and Studier (1973) to electrophorese proteins and isozymes.

For Statistical analysis a completely randomized design was used, and mean comparisons were made using Duncan's Multiple Range test at 5% significant level (Duncan 1955).

3. RESULTS AND DISCUSSION

The studied banana cultivars exhibited differences in radio sensitivity. Radio sensitivity was assessed as percentage of survival, number of the proliferated shoots, shoot length and wet and dry weights of the proliferated shoots (Table 1). Survival rate of `Grand Nain' was decreased with increasing gamma irradiation dose up to 60 Gy, survival rate of `Gros Michel' remained stable with increasing gamma irradiation dose. However survival rate of `Williams' was stable after irradiation with gamma at 40 Gy, a higher gamma irradiation dose (60 Gy) reduced survival rate.

Shoot proliferation was affected by gamma irradiation treatments in the three banana cultivars (Table 1). The number of proliferated shoots was decreased as gamma irradiation dose increased. Cultivars differed in the degree of shoot number reduction in response to gamma irradiation. At low gamma irradiation dose (40 Gy) shoot number was reduced by 65.5, 61.8 and 54.3 % in `Gros Michel', `Williams' and `Grand Nain', respectively. At high gamma irradiation dose (60 Gy), no significant differences in the shoot number reduction were found among the three cultivars.

Cultivars differed in shoot length changes in response to gamma irradiation (Table 1). Gamma irradiation had no significant effect on shoot length of `Grand Nain'. Shoot elongation of `Gros Michel' however, decreased with increasing gamma irradiation dose to 60 Gy. Gamma irradiation at 40 Gy increased shoot length of `Williams'. Higher dose of irradiation (60 Gy) decreased shoot length compared with mother "Williams". Exposure to gamma irradiation *in vitro* banana shoot tissues led to retardation of shoot growth, leaf deformities and chlorophyll streaking (Espino *et al.* 1986). The high doses of gamma irradiation gave rise to more plants showing chlorosis and necrosis (Silayoi *et al.* 1986).

Table 1. Effect of gamma irradiation (Gy) on survival rate and shoot development of three banana genotypes.

Cultivars	Survival %			Shoot number			Shoot length (cm)		
				Irradiation (Gy)					
	0	40	60	0	40	60	0	40	60
Grand Nain	100	95	85	18.5Aa	8.4Ab	4.0Bc	2.18Ba	2.36Aa	2.54Aa
Gros Michel	100	100	100	19.9Aa	6.9Bb	4.6Ac	3.12Aa	2.49Ab	1.08Bc
Williams	100	100	95	14.1Ba	5.4Cb	3.2Cc	1.37Bb	2.04Aaq	1.12Bb

Mean separation by Duncan's multiple range. $P \leq 0.05$.
Big letters for cultivars and small letters for treatments.

Table 2. Effect of gamma irradiation on shoot weights (g) of three banana genotypes.

Cultivars	Wet weight			Dry weight			Wet weight/Dry weight		
				Irradiation (Gy)					
	0	40	60	0	40	60	0	40	60
Grand Nain	10.21Aa	3.83Ab	2.57Ac	0.40Aa	0.27Ab	0.21Ac	25.5	14.2	12.2
Gros Michel	7.40Ba	3.36Ab	2.69Ac	0.31Aa	0.24Ab	0.20Ac	23.9	14.0	13.5
Williams	2.77Ca	1.19Bb	0.91Bc	0.19Ba	0.09Bb	0.07Bc	14.6	13.2	13.0

Mean separation by Duncan's multiple range. $P \leq 0.05$.
Big letters for cultivars and small letters for treatments.

Table 3. Effect of NaCl in the media on the survival rate and shoot development of 'Williams' salt-tolerant mutant line.

Tissue type	Survival %				Shoot number				Shoot length (cm)			
					NaCl%							
	0.00	0.25	0.75	1.25	0.00	0.25	0.75	1.25	0.00	0.25	0.75	1.25
Mutant line	100	100	100	15	14.2Aa	14.5Aa	5.1Ab	1.0c	1.38Ab	1.54Bab	1.65Aa	1.15c
Mother	100	85	75	0	14.1Aa	7.1Bb	3.2Bb	--	1.28Ab	2.98Aa	1.00Bb	--

Mean separation by Duncan's multiple range. $P \leq 0.05$.
Big letters for cultivars and small letters for treatments.

Table 4. Effect of NaCl in the media on tissue weights of 'Williams' salt-tolerant mutant line.

Tissue type	Wet weight				Dry weight				Wet weight/ Dry weight			
					NaCl%							
	0.00	0.25	0.75	1.25	0.00	0.25	0.75	1.25	0.00	0.25	0.75	1.25
Mutant line	2.79Aa	2.67Aa	0.87Ab	0.46c	0.19Aa	0.19Aa	0.09Ab	0.05c	14.7	14.1	9.7	9.2
Mother	2.27Aa	1.90Ba	0.71Bb	---	0.18Aa	0.10Bab	0.05Bb	---	15.3	19.0	14.2	--

Mean separation by Duncan's multiple range. $P \leq 0.05$.
Big letters for cultivars and small letters for treatments.

Wet weight of cultures in the three cultivars was affected by gamma irradiation treatments (Table 2). Wet weight decreased as gamma irradiation dose increased. Cultivars differed in the degree of reduction in culture wet weight in response to gamma irradiation. The relative wet weight reduction in `Grand Nain' was higher than that in `Gros Michel' and `Williams' at both gamma irradiation doses. Novak *et al.* (1990) reported that radio sensitivity differed among cultivars and was assessed as fresh weight gain and rate of shoot proliferation.

Gamma irradiation reduced shoot dry weight of the three cultivars (Table 2). Low gamma irradiation dose (40 Gy) decreased wet weight/dry weight ratio. No more reduction was observed with increasing gamma irradiation.

The three banana cultivars differed in the reduction of growth in response to gamma irradiation. `Grand Nain' expressed the highest level of radiation damage of the tested cultivars, suggesting that `Grand Nain' is the most sensitive to gamma irradiation. Such intercultivar differences in radio sensitivity were noted in banana (Espino *et al.* 1986, Novak *et al.* 1990, 1993) and rice (Liang *et al.* 1988).

Mutagenic treatments that were applied to tissues caused morphological variants, the most obvious one is albino; that is reduced chlorophyll content in some leaf cells to the level that make tissues seem white. This was obtained in a chimeric `Williams' clone derived from tissues treated with 40 Gy gamma irradiation (Fig 1). Considerable phenotypic variations in banana were observed among plants regenerated from *in vitro* shoot tips after gamma irradiation treatment (Novak *et al.* 1990). One of the arisen variants was a chimaera (Novak and Mike 1988).

Inducing mutations *in vitro* allowed the quick and efficient screening of a large number of mutant plants (Domingues *et al.* 1994). A mutation frequency was found 300 times greater than the natural frequency in citrus callus cultured *in vitro* which was exposed to gamma irradiation (Wan *et al.* 1991). Chromosome aberrations, such as laggards, fragments and bridges, were induced by gamma irradiation in callus cells of citrus (Deng *et al.* 1989). In banana, gamma irradiation was used to produce mutant lines superior in different traits among which is resistance to *Fusarium oxysporum*

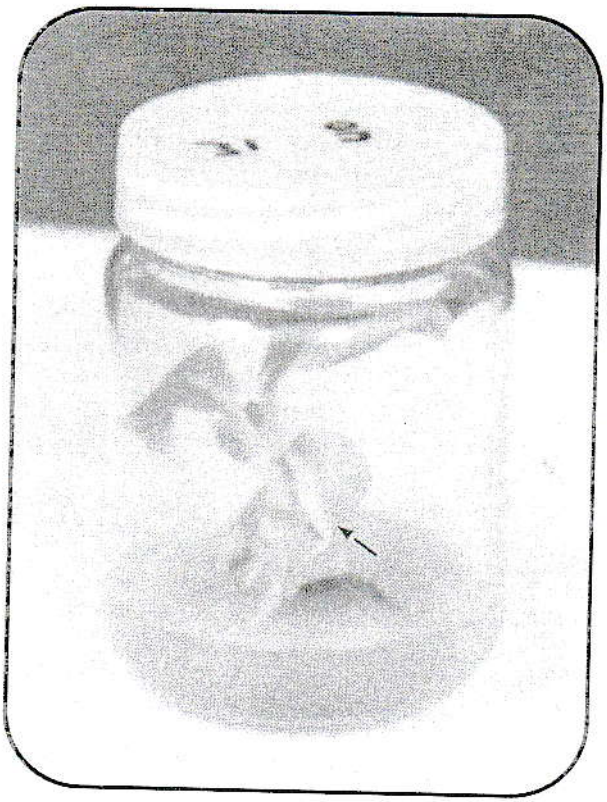


Fig 1. Chimeric 'Williams' plant derived from tissues irradiated with 40 Gy gamma rays.

(Tulmann-Neto *et al.* 1990), improving cold tolerance (Smith *et al.* 1993) and early-flowering (Novak *et al.* 1990, Tan *et al.* 1993).

Lines derived from irradiated tissues of `Grand Nain' and `Gros Michel' exhibited no significant differences in growth traits compared with the mother tissues in media contained 0.25, 0.75 or 1.25% NaCl. Among gamma irradiated tested lines of `Williams', one mutant line showed enhancement of growth traits compared with the mother tissues under salt stress (Tables 3, 4 and Fig.2). This line was obtained by 40 Gy gamma irradiation and was able to survive 1.25% NaCl. Survival rate of `Williams' salt-tolerant mutant line remained stable with increasing the concentration of NaCl in the media up to 0.75%. Fifteen percent of `Williams' salt–tolerant mutant line explants survived NaCl at 1.25% (Table 3). While the survival rate of the mother `Williams' declined linearly with increasing NaCl concentration up to 0.75%, a higher concentration of NaCl (1.25%) caused the death of all explants.

NaCl at 0.25% had no effect on shoot number of `Williams' salt-tolerant mutant line. On the other hand, shoot proliferation in the mother `Williams' was adversely affected by NaCl at 0.25%, as number of shoots was reduced to 50% comparing with those of the untreated culture (Table 3). A sudden decrease in the number of shoots per explant at 0.75% NaCl was observed in both tissues. The reduction in shoot number of the mother `Williams' was greater than that in `Williams' salt-tolerant mutant line.

NaCl at 0.25% had no effect on shoot length of `Williams' salt-tolerant mutant line, however NaCl at 0.75% increased the shoot length (Table 3). On the other hand, with the mother `Williams', while low concentration of NaCl (0.25%) increased the shoot length, high concentration (0.75%) decreased shoot length back to control level.

No reduction was noticed in shoot wet weight of `Williams' salt-tolerant mutant line or with the mother `Williams' in response to 0.25% NaCl (Table 4). The two tissue types showed a sudden decrease in wet weight at 0.75% NaCl. The reduction in shoot wet weight of the mother `Williams' was more than that in `Williams' salt-tolerant mutant line grown in media containing 0.75% NaCl.

NaCl at 0.25% had no effect on reducing shoot dry weight in the two tissue types (Table 4). However, higher NaCl concentration (0.75 %) reduced shoot dry weight of the two tissue types. The

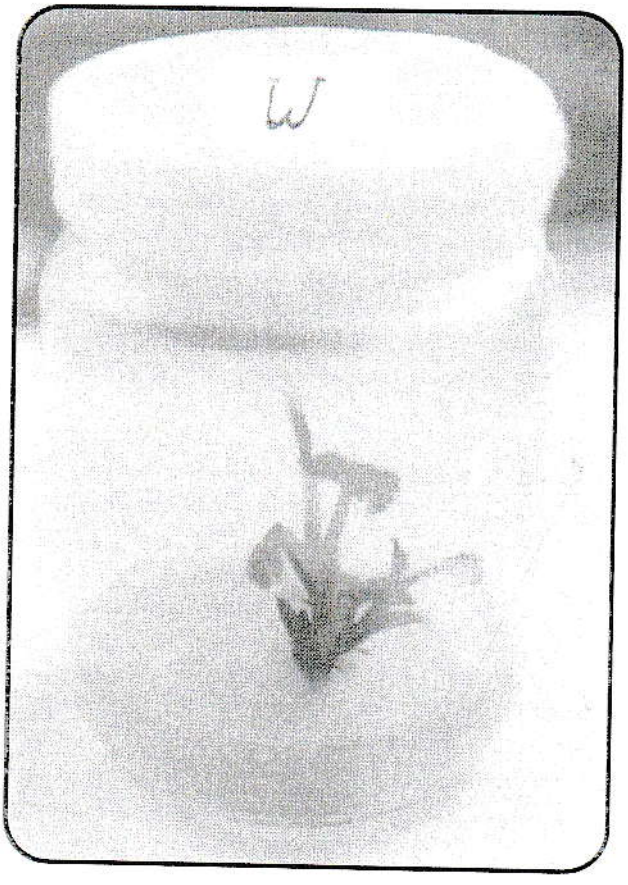


Fig 2. The salt-tolerant mutant line derived from 'Williams' tissues irradiated with 40 Gy gamma rays.

reduction in dry weight in response to salt stress in the mother 'Williams' was higher than that in the salt-tolerant mutant line. Low concentration of NaCl (0.25%) had no effect on wet weight/dry weight ratio. However the higher concentration decreased wet weight/dry weight ratios of both tissues (Table 4).

A mutant cell line, obtained in citrus by gamma irradiation, was able to survive on 8% NaCl (Deng *et al.* 1989). Other two salt-resistant mutants were obtained after treating citrus callus with gamma irradiations and screening with NaCl *in vitro* (Wan *et al.* 1991). Calluses produced from kiwifruit explants treated with gamma irradiation were selected for NaCl tolerance (Wang *et al.* 1990). Gamma irradiation was useful in screening for NaCl tolerance in wheat (Xaio and Zhao 1989), rice (Liang *et al.* 1988), English ivy (Brawley and Mathes 1990).

The salt-tolerant mutant line of 'Williams' showed absence of one protein of 146.5 KD MW that characterized the mother 'Williams' and induced three other proteins of 43.2, 70.0 and 129.3 KD MW (Table 5 and Fig.3). One possible explanation for the completely disappearance of the 146.5 KD protein is that the gene (s) responsible for a certain protein had been completely inhibited as a result of irradiation of the mother explant. Therefore, the developed mutant had lost its ability to synthesize this protein. Leaf protein electrophoresis of early-flowering putative mutant plant of 'Grand Nain' provided evidence of an altered genetic nature of the mutant plant in comparison with the mother clone. The mutant showed a less dense staining protein of 33KD MW and the absense of three other proteins , characterized the mother 'Grand Nain' (Novak *et al.* 1990, 1993).

A biochemical assay could aid in early screening and in the assessment of genetic nature of phenotypic changes (Novak *et al.* 1990). Methods are being developed to detect somaclonal variants of *Musa* in the nursery and in the field, but what is needed are early-stage markers that can be applied *in vitro* to detect variation as soon as it occurs (Withers 1993). Investigation of the properties of the newly formed proteins that occurred in salinity, and knowledge of their functions will undoubtedly provide useful information in understanding the mechanism of salt-tolerance and for selection of tolerant mutants (Liu and Li 1991).

Table 5. Differences between the salt-tolerant mutant line and the mother 'Williams' in leaf SDS-extractable protein distribution.

Band No.	MW* (KD)	Tissue type		Band No.	MW* (KD)	Tissue type	
		Mutant	Mother			Mutant	Mother
1	146.5	-	+	23	33.8	+	+
2	131.6	+	+	24	32.0	+	+
3	129.3	+	-	25	31.8	+	+
4	127.0	+	+	26	30.0	+	+
5	125.6	+	+	27	29.8	+	+
6	112.3	+	+	28	29.2	+	+
7	99.1	+	+	29	28.8	+	+
8	88.8	+	+	30	28.5	+	+
9	82.7	+	+	31	28.4	+	+
10	70.0	+	-	32	24.6	+	+
11	65.4	+	+	33	24.4	+	+
12	59.7	+	+	34	24.2	+	+
13	55.5	+	+	35	24.1	+	+
14	51.0	+	+	36	23.3	+	+
15	44.9	+	+	37	21.1	+	+
16	43.2	+	-	38	19.7	+	+
17	42.7	+	+	39	18.7	+	+
18	40.9	+	+	40	18.5	+	+
19	39.8	+	+	41	16.6	+	+
20	38.1	+	+	42	14.6	+	+
21	37.8	+	+	43	14.0	+	+
22	35.8	+	+				

+ = Present, - = Absent

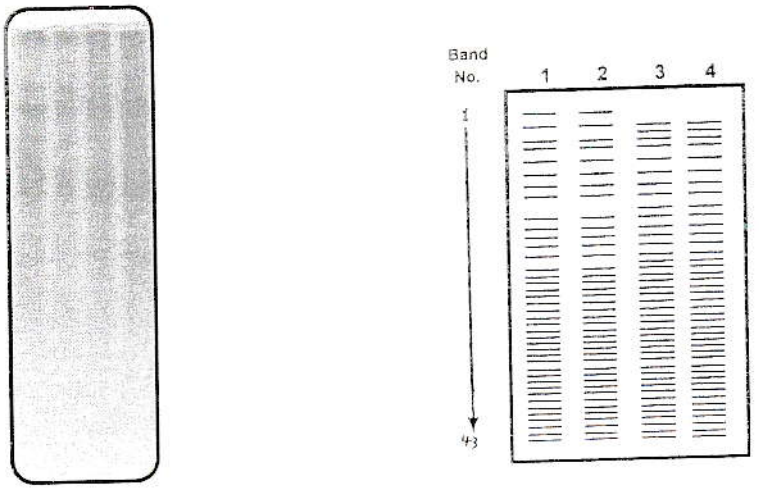


Fig 3. Patterns of leaf SDS-extractable proteins of the salt tolerant mutant line (lane 3, 4) and the original 'Williams' (lane 1, 2).

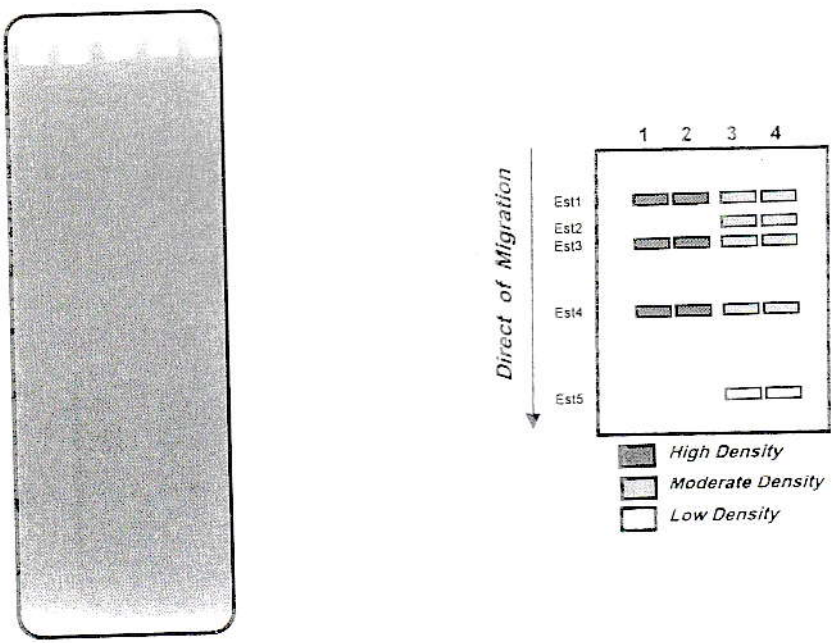


Fig 4. Electrophoretic patterns of esterase isozymes for the salt tolerant mutant line (lane 3, 4) and the original 'Williams' (lane 1, 2).

Table 6 :Differences between the salt-tolerant mutant line and the mother `Williams' in distribution of esterase groups according to the relative mobility and density.

	Esterase group				
Tissue type	Est1	Est2	Est3	Est4	Est5
	Relative mobility				
	0.18	0.23	0.25	0.36	0.78
Mutant line	**	**	**	**	*
Mother	***	-	***	***	-

*** = High density ** = Moderate density * = Low density - = Absent

Table 7. Differences between the salt-tolerant mutant line and the mother `Williams' in distribution of peroxidase groups according to the relative mobility and density.

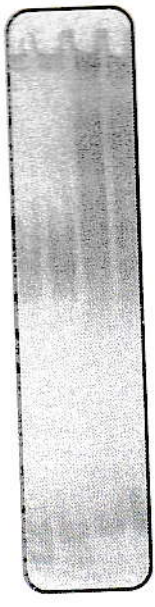
	Peroxidase group							
Tissue type	Px1	Px2	Px3	Px4	Px5	Px6	Px7	Px8
	Relative mobility							
	0.02	0.11	0.19	0.21	0.60	0.62	0.64	0.66
Mutant line	**	***	**	***	**	**	-	-
Mother	-	-	**	***	-	***	***	**

*** = High density ** = Moderate density * = Low density - = Absent

Table 8. Differences between salt-tolerant mutant line and mother `Williams' in distribution of malate dehydrogenase groups according to the relative mobility and density.

	Malate dehydrogenase group						
Tissue type	Mdh1	Mdh2	Mdh3	Mdh4	Mdh5	Mdh6	Mdh7
	Relative mobility						
	0.05	0.14	0.25	0.37	0.52	0.67	0.79
Mutant line	*	*	*	*	*	*	*
Mother	-	-	-	**	**	**	**

*** = High density ** = Moderate density * = Low density - = Absent



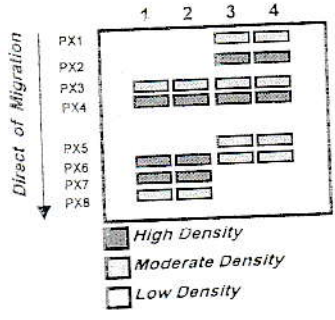
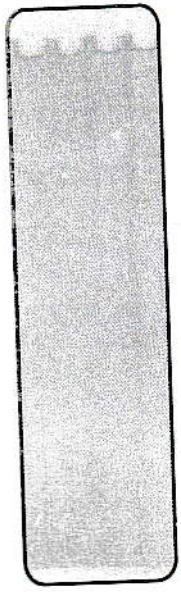


Fig 5. Electrophoretic patterns of peroxidase isozymes for the salt tolerant mutant line (lane 3, 4) and the original 'Williams' (lane 1, 2).



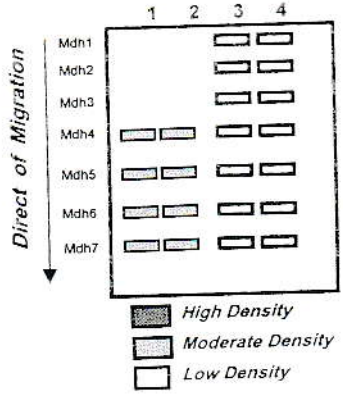


Fig 6. Electrophoretic patterns of malate dehydrohenese isozymes for the salt tolerant mutant line (lane 3, 4) and the original 'Williams' (lane 1, 2).

The results of SDS electrophoresis indicated that the number of detectable bands of soluble proteins from NaCl-tolerant *Setaria italica* callus explants was higher than that from the control calluses. Two new bands of 59 and 90 KD proteins were detected in salt-tolerant callus, but were absent in the controls (Jia *et al.* 1993). SDS-PAGE revealed that the salt-tolerant somaclones derived from salt-tolerant maize cultivar Arizona 8601 had protein patterns similar to those of Arizona 8601 but different to those of the salt sensitive genotypes (Lupotto *et al.* 1990).

The electrophoretic patterns of esterase isozymes showed differences between the salt-tolerant mutant line and the mother `Williams' (Table 6 and Fig. 4). The salt-tolerant line showed a less densely staining isozymes of 0.18, 0.25 and 0.36 relative mobility that characterized the mother `Williams' and induced two other bands of esterase isozymes with relative mobility of 0.23 and 0.78. The early flowering putative plant of `Grand Nain' showed the absense of two bands of esterase isozymes that characterized the mother `Grand Nain' (Novak *et al.* 1990, 1993).

The electrophoretic patterns of peroxidase isozymes showed differences between the salt-tolerant mutant line and the mother `Williams' (Table 7 and Fig. 5). The salt-tolerant line showed a less densely staining isozymes of 0.62 relative mobility and lack of two other isozymes with relative mobility of 0.64 and 0.66 that characterized the mother `Williams' and induced three other bands of peroxidase isozymes with relative mobility of 0.02, 0.11 and 0.60. The peroxidase isozymes of the resistant callus and leaves of the regenerated tobacco plants were different from those in the mother material (Zhou and Yang 1986).

The electrophoretic patterns of malate dehydrogenase isozymes showed differences between the salt-tolerant mutant line and the mother `Williams' (Table 8 and Fig.6). The salt-tolerant line showed a less densely staining malate dehydrogenase isozymes of 0.37, 0.52, 0.67 and 0.79 relative mobility that characterized the mother `Williams' and induced three other bands of malate dehydrogenase isozymes with relative mobility of 0.05, 0.14 and 0.25. With Shamouti orange, cell lines were selected from ovular callus. The salt-tolerant line was found to be a true cell line variant and showed the highest malate dehydrogenase activity. Electrophoretic patterns of Malate

dehydrogenase showed three isozymes for the salt-tolerant line, but only one for the other lines (Libal *et al.* 1985). The reduction of the number of polymorphic bands on a zymogram corresponded to a deletion or inhibition of a specific allele in the enzyme locus (Novak 1990). Such genetic interpretation has been made several times in different plant species (Shield *et al.* 1983).

Salt stress is one of the most important factors affecting growth and yield of banana. Developing salt tolerant germplasm will encourage banana cultivation in salty areas. *In vitro* induction of mutations and selection methods were employed to select banana cell culture capable of growing in the presence of salt. Gamma irradiation was found to be a useful tool in inducing mutations for salt-tolerance. Tissue culture techniques were utilized successfully for selection of salt-tolerant mutants. The system used allowed the quick and efficient screening for salt-tolerance.

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الإستحداث والإنتخاب للموز المتحمل للملوحة فى الأنبوب

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ملخص

أستخدمت أشعة جاما كوسيلة مطفرة للموز في الأنبوب واستخدم تكنيك زراعة الأنسجة لانتخاب الطفرات المتحملة للملوحة. فبعد زراعة ثانوية مباشرة عرضت الأجزاء النباتية من الأصناف جراند نان، المغربى والوليامز لأشعة جاما بالجرعات ٤٠ أو ٦٠ جراى وذلك باستخدام مشعع ميجا جاما جا ٦٦٠٠ كوبالت. اختبرت الأصناف فى مدى حساسيتها للإشعاع عن طريق تقدير نسبة الأجزاء الحية، عدد الأفرع ، طول الأفرع والوزن الطازج والوزن الجاف للنموات الناتجة. ولقد أظهرت أصناف الموز اختلافات فى مدى حساسيتها للإشعاع. أدت معاملات الإشعاع إلى ظهور تباينات مظهرية ولعل أكثرها وضوحاً الألبينو. وللإنتخاب لتحمل الملوحة بعد معاملات الإشعاع، أعتبر كل جزء نباتى مشعع كأم لخط مستقل. ولقد اختبرت الأفرع الناتجة من كل خط لتحمل الملوحة في بيئات تحتوى على كلوريد صوديوم بتركيز ٠,٢٥ ، ٠,٧٥ و ١,٢٥%. وجد من بين الخطوط المختبرة للصنف الوليامز خط مطفر يظهر نمو أفضل منه فى الأنسجة الأم وذلك

في وجود مستويات الملوحة المذكورة. ولقد تم الحصول على هـذا الخـط بعـد المعاملة بالجرعة ٤٠ جراى من أشعة جاما. لم تقل نسبة الأجزاء التى استمـرت حية متحملة كلوريد الصوديوم حتى ٠,٧٥% . بينما تحملت ١٥% من أجزاء هذا الخط كلوريد الصوديوم بتركيز ١,٢٥%. لم يكن لكلوريـد الصوديـوم بتركـيز ٠,٢٥% أى تأثير على عدد الأفرع لهذا الخط، ومع ذلك فلقد حدث نقص فجـائى فيها عند تركيز ٠,٧٥%. لم يتأثر طول الأفرع لهذا الخط بكلوريد الصوديوم عند ٠,٢٥%، بينما زاد عند تركيز ٠,٧٥% منه. لم يلاحظ نقص في الوزن الطازج لهذا الخط استجابة لكلوريد الصوديوم بتركيز ٠,٢٥%. وكان النقص فى الـوزن الطازج للأنسجة الأم أكبر منه فى هذا الخط عندما نمت في بيئـة تحتـوى علـى كلوريد صوديوم بتركيز ٠,٧٥%. درسـت التغـيرات فـي تخليـق الـبروتين والمشابهات الإنزيمية لتوضيح ميكانيكية تحمل الملوحة فى هذا الخـط المتحمـل للملوحة. أظهرت أنسجة هذا الخط غياب أحد البروتينات ذات الـوزن الجزيئـى ١٤٦,٥ كيلودالتون والتى تميز الأنسجة الأم لصنف الوليامز. كما ظهرت ثـلاث بروتينات جديدة ذات وزن جزيئى ٤٣,٢ ، ٧٠,٠ و ١٢٩,٣ كيلو دالتون. أظـهر التحليل الكهربى للمشابهات الإنزيمية اختلافات بيـن الخـط المتحمـل للملوحـة والأنسجة الأم.