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EFFECT OF UV AND MICROWAVE TREATMENTS ON *IN VITRO* GROWN *YUCCA ELEPHANTIPES* MEDICUS

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ABSTRACT

Yucca plants were exposed to microwave and ultraviolet rays, using the doses 0, 1, 3 and 6 (0, 95, 195 and 390 watt for period 2, 4 and 8 seconds). Ultraviolet (80 waves) rays for 2, 4 and 6 hours to induced genetical variations. Irradiated explants cultured on Murashige and Skoog medium supplemented with 6-Benzylaminopurine (BAP) 2.6 mgIL and Indole-acetic acid (IAA) 1.25mg/L were suitable for direct regeneration. Microwave rays were more effective than ultraviolet in maximizing of somaclonal variation. Vegetative parameters of regenerated plantlets were affected by the used mutagen .In the first sub- culture, exposure of explant to microwave at power 6 for 8 seconds significantly increased plant height and number of shoots per plant .All treatments increased number of leaves, as compared with non- treated (control) while subjecting to power 3 for 2 seconds gave the maximum callus production .In the second sub culture all doses of microwave and ultraviolet treatments significantly reduced plant height .So exposure of explants to microwave of power 1 for 2,4 and 8 seconds and UV for 4 and 6 hours significantly increased number of leaves ,while subjecting to power 1 for 8 second increased number of leaves and callus production .Leaf area was significantly increased with microwave of power 1 for 4 second, power 3 and 6 for 8 seconds and UV for 4 and 6 hours . Chlorophyll a and b and carotenoids were significantly increased with microwave at 1 and 3 power for 8 seconds compared with control. Polyphenyl Oxidase (PPO) and Peroxidase (PRX) isozyme patterns were influenced by the used mutagen. Moreover 0, 1, 3 and 6 (95, 195 and 390 watt for period 2, 4 and 8 seconds) and ultraviolet (80 waves) rays for 2, 4 and 6 hours were effective doses which induced genetical variations.

Key Words: ***Yucca, Mutation, UV, Microwave, Chlorophyll, Isozymes.***

INTRODUCTION

Yucca plant (*Yucca elephantipes*, Medicus, Family: Agavaceae) is one of the most important ornamental shrubs plants in warm regions. It is showy flowered ever greens of impressive decorative and suitable for lawn planting and subtropical massing. This plant can be grown as indoor or outdoor attractive plants. It tolerates different climatic conditions; *i.e.* wet and dry weather, high and relatively low temperature. The conventional propagation methods of yucca plants were practiced by seeds, stem cuttings or by rhizome cuttings, in recent years *in vitro* regeneration has become the most common method (Youssef 2003).

Plant breeding requires genetic variation of useful traits for crop improvement. Often, however, desired variation is lacking. Mutagenic agents such as ionizing radiation and certain chemicals can be used to induce mutations and generate genetic variations from which desired mutants may be selected.

Tissue culture is a potential tool for clonal propagation. However, before one can make use of this technique for mutation induction purposes, there is a need to establish proper protocol for plantlet regeneration

Induced mutation holds promise in the genetic improvement of plants. In crops that do not produce any seed, mutation breeding is the only way to develop new improved varieties; it is also the sole method to induce the desired character which is not present in the existing clone.

Mutation breeding is suitable for the breeding of ornamentals plants because the new mutant varieties and the original ones have the same genetic background except for the mutated gene. This implies that the original and the new cultivars (mutants) may be produced under the same culture conditions, while new cultivars obtained *via* cross breeding might require different growing regimes according to the reviews of Lernes (1928). In gladiolus when the meristems were cut from the corm irradiated with gamma rays and then cultured *in vitro,* the plantlets could be regenerated *via* embryogenesis and showed a high frequency of variants without chimeras (Kasumi *et al* 2001)

Radiation-induced mutations, in combination with tissue culture techniques greatly enhance the efficiency of mutation breeding. In *vitro* effect of irradiation on a number of flower species has been reported by Youssef (1998); Nandanwar and Patil (2000) and Youssef (2003).

The objective of this investigation was to induce genetic variation in yucca through different irradiation mutagens and using *in vitro* technique . These genetic variations could be used by plant breeders for selection of useful traits.

MATERIALS AND METHODS

This investigation was carried out at the Tissue Culture and Germoplasm Conservation Research Laboratory, Horticulture Research Institute, Giza, Egypt;during (2006/2007) season in order to investigate the influence of different powers for several times of microwave and various doses of ultraviolet on the in vitro micropropagability of explants (during two consecutive micropropagation stages of shooting and rooting) and their biochemical constituents.

***In vitro* culture** homogeneous and healthy plants of yucca were grown in Horticulture Research Institute. The shoot tip (the apical dome with 3- 4 leaf primordia) of one node cuttings were taken. Explants were sterilized in 15% Cholorax solution containing two drops of Tween-20 for 15 minutes then transferred to 70% ethyl alcohol for 5 minutes. After that explants were washed 3 times thoroughly with sterilized distilled water. Then explants were plated in glass vials each contained 40 ml of Ms medium.

The medium consists of Murashige and Skoog (1962) salts and supplemented with 30 g/L sucrose, BAP 2.6 mg/L , IAA 1.25 mg/L and 7.0 g/L agar and PH was adjusted to 5.7.The medium was autoclaved at 121°C for 20 minutes. Cultured vials ware incubated in growth chamber at 27±1°C under 16 hr light and 8 hr dark.

Microwave and UV treatments

The *in vitro* explants were microwaved for 2, 4 and 8 seconds by 0, 1, 3 and 6 watt. The used microwave apparatus is a single phase with grounding 1.3 k.w. output 650 at a frequency of 2450 MH2 (Model#Mo6T, 22 v-soH2).

So the *in vitro* explants were irradiation by ultraviolet (80 watts) rays for 2, 4 and 6 hours.

Twenty-five explants in five replicates were used for each one of microwave and ultraviolet treatments. In the first cycle of subculture, the explants cultures were incubated for 2 month and the obtained shootlet were aseptically divided into explants and recultured in the second cycle of subculture on fresh medium for after 8 weeks.

At the end of culture periods, the developmental microcutings were submitted to study wach of the *in vitro* micropropagability of explants and their biochemical analysis procedures.

Data and Calculation

Callus production (referred to the plantlets growth), these scores were given as follow: Negative results =1, below average =2, average =3, above average =4, and excellent =5 (according to Pottino 1981), plant height (deals with stem length), leaf area in cm^2 by c1-203 AREA METER , CID, Tnc number of shoots , number of leaves ,rooting (recorded as number of roots developed), root length (deals with root length) and survival capacity of explant was established on percentage.

Plant chemical compositions

Chlorophyll a, b and carotenoides mg/g were determined in the leaves of plants according to Saric *et al* (1967).

Isozyme analysis:

Young leaves of yucca were collected and kept at -20°C. Leaf extracts were prepared by grinding 200 mg of fresh leaves in 1.5 ml of the following extraction buffer: KH_2PO_4 (0.1 M), sucrose (7%), ascorbic acid (0.4%),β-mercaptoethanol (0.15%), polyvinylpolyrolidone (8%), pH:7.5 and centrifuged at 14.000 rpm for 30 min.Electrophoresis was performed in acrylamide gels for peroxidase at EC (1.11.1.7) and EC (3.4.11.1) for Polyphenyl Oxidase according to (Chevreau *et al* 1997).

Statistical analysis

Each treatment included five gars each gar contained four explants and each experiment was replicated three times. Data were subjected to analysis of variance by MSTAT-C (1990) Computer statistical analysis program. LSD, test at the 5% level of significance (P=0.05) was computed to differentiate between means.

RESULTS AND DISCUSSION

Plant regeneration was observed on the explants cultured on the tested medium although their effectiveness varied significantly depending on inducing mutations and genetical variations by using different doses of microwave and ultraviolet rays (Tables 1, 2, and 3 and Figs 1, 2 and 3).

Effect of irradiation treatments of *Yucca elephantipes* in the first culture

Data in Table (1) see also (Fig 1 and photo1) showed the effect of different doses of microwave and ultraviolet rays treatments on Yucca characteristics. Data showed direct effect of microwave and ultraviolet rays' treatments on plant height, number of shoots, number of leaves and callus production. The highest dose (powers 3&6 for 8 seconds) induced significant increase in plant height. Plant height was 3.40 cm and 4.00 cm as compared with the control (3.00 cm). Also it was clear that the higher dose of microwave (power 6 for 8 seconds) induced the non significantly highest number of shoots per plant (3.00 as a compared 2.33with for control). The number of leaves per plant was 8.33, 8.00 and 11.00, respectively as compared with the control (5.66), The lowest dose (power 3 for 2 seconds) produced callus production (2.00) as compared with control (1.00). On the other hand 4 hours ultraviolet rays' treatment induced significant reduction in plant height (2.50 cm). However a significant increase was observed in number of shoots per plant (3.00), number of leaves / plant (8.00), callus production (1.00) as a compared with control, which was 3.00, 2.33, 5.66, 1.00, respectively. Application of different microwave treatments caused a significant reduction in survival capacity (to 40%)of regenerated plants. When using power 3 for 3 seconds power 6 for 2 seconds and power 6 for 8 seconds as microwave treatments and 4 hours UV treatment. Similar results were reported by Nagatomi *el al* (1998) Youssef (2003) and Sayed *et al* (2005).

Table 1. Effect of irradiation for different periods by microwave and UV on growth of *Yucca* in the first culture

Treatment (dose) in (watt)	Exposure time	Plant height (cm)	Number of shoots	Number of leaves/shoot	Callus production (scores)	Survival %
Microwave						
control	0.00	3.00	2.33	5.66	1.00	100
1	2 Sec	3.00	1.33	7.66	1.33	100
1	4 Sec	2.34	1.66	8.33	1.00	100
1`	8 Sec	2.16	1.66	8.33	1.33	100
3	2 Sec	3.00	1.00	6.00	2.00	40
3	4 Sec	2.23	2.00	7.00	1.33	100
3	8 Sec	3.40	1.00	8.00	1.33	100
6	2 Sec	2.00	2.00	7.00	1.00	40
6	4 Sec	3.50	2.33	11.00	1.00	100
6	8 Sec	4.00	3.00	7.00	1.00	40
U.V.						
80	2 hr	2.50	2.00	7.00	1.00	100
80	4 hr	2.50	3.00	8.00	1.00	40
80	6 hr	2.33	2.66	7.33	1.00	100
$LSD_{0.5}$		0.68	N.S	N. S	0.005	

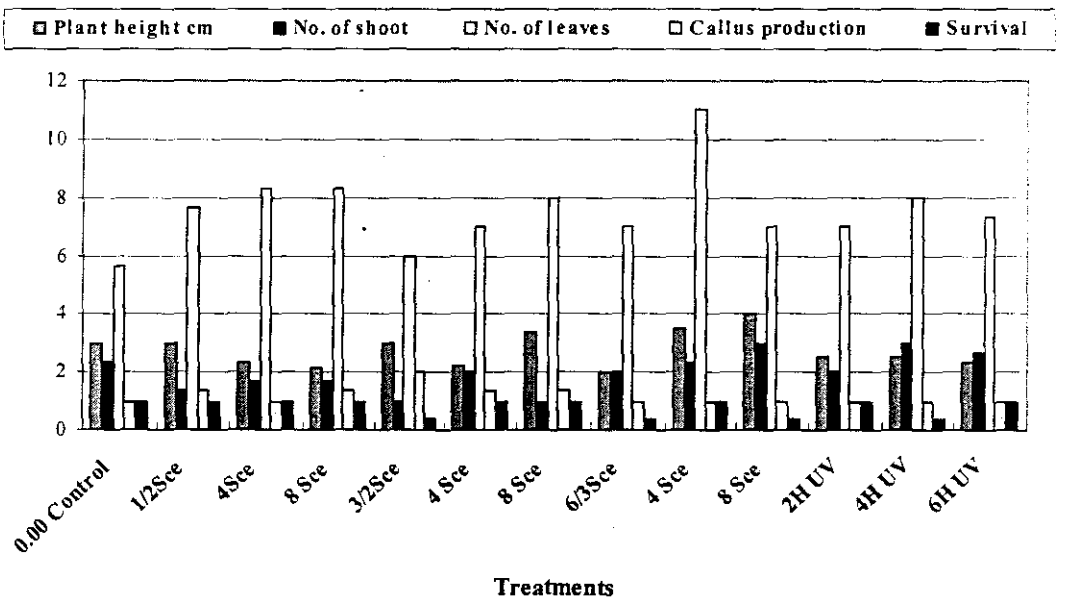

Fig. 1. Histogram showing the effect of irradiation for different periods by microwave and UV on growth of yucca in the first culture

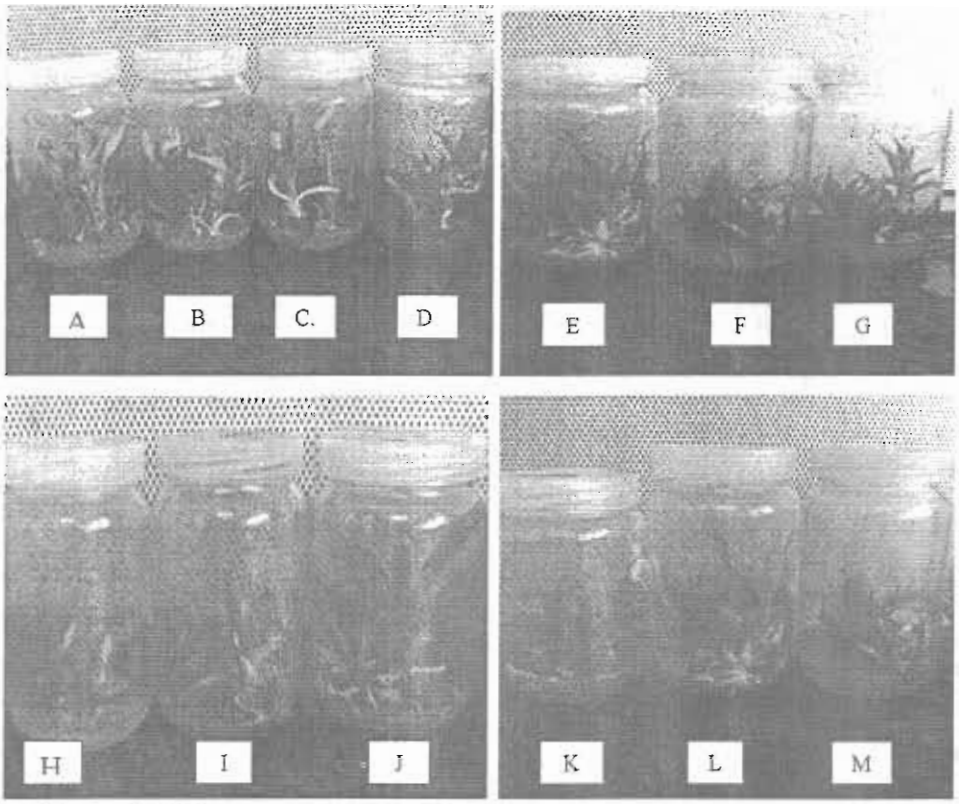

Photo 1. Effect of different period in microwave and UV ray on growth parameters of yucca plant A=Con ,power [1] B= 2 Sec, C=4Sec, D=8 Sec ,power [3]E =2 Sec, F=4 Sec, G=8 Sec ,power [6] H= 2Sec, I=4 Sec, J=8 Sec and UV ray K= 2 hr, L=4 hr, M=6hr.

Effect of irradiation treatments in the second culture on yucca characteristics.

The results in Table (2) and (Fig. 2) showed the effect of irradiation by microwave and ultraviolet rays' treatments for different periods on plant height, number of shoots, number of leaves, callus production, leaf area, number and length of roots. It is clear that microwave and UV treatments induced significantly reduction in plant height, it was 3.83 cm, 3.50 cm, 3.75 cm, 4.00 cm and 4.00 cm for 1,3 and 6 microwave power with 8 seconds and 4 hours UV ,respectively in comparison with the control (4.75 cm) significant increase was observed in number of shoots; it was 11.00 (in power 1 for 2 seconds), 8.33(in power 6 for 2 seconds) and 12.33(in 4 hours) UV, in comparison with the control (7.66). The number of leaves was significantly increased; it was 9.00 (in power 1 for 8 seconds) in comparison.

Table 2. Effect of irradiation for different periods by microwave and UV on growth of Yucca in second culture

Treatment dose in (watt)	Exposure time	Plant height (cm)	Number of shoots	Number of leaves Shoot	Callus production (scores)	Leaf area cm^2	Number of roots	Roots length (cm)	Survival %
Microwave									
control	0.00	4.75	7.66	8.00	1.00	1.76	3.33	9.33	80
1	2 Sec	2.35	11.00	6.00	1.00	1.75	2.33	2.25	80
1	4 Sec	3.50	10.00	7.66	1.00	2.26	5.66	8.00	100
1	8 Sec	3.83	8.00	9.00	2.00	1.01	3.66	7.16	80
3	2 Sec	3.50	3.33	6.66	1.00	0.60	10.33	15.50	80
3	4 Sec	2.00	5.00	6.66	1.00	1.06	7.50	4.00	80
3	8 Sec	2.50	7.00	7.00	1.00	2.00	1.00	1.16	100
6	2 Sec	3.75	8.33	8.00	1.00	0.90	13.00	15.50	80
6	4 Sec	3.16	4.00	5.66	1.00	1.56	4.00	7.50	100
6	8 Sec	2.00	4.00	5.00	1.00	3.00	2.00	2.00	40
UV									
80	2 h	2.66	5.66	6.33	1.00	1.08	1.66	2.33	100
80	4 h	4.00	12.00	7.00	1.00	1.50	9.00	12.00	40
80	6 h	3.00	12.33	6.66	1.00	2.46	6.66	10.00	100
$LSD_{0.5}$		62	4.10	1.34	1.75	0.95	2.25	3.62	

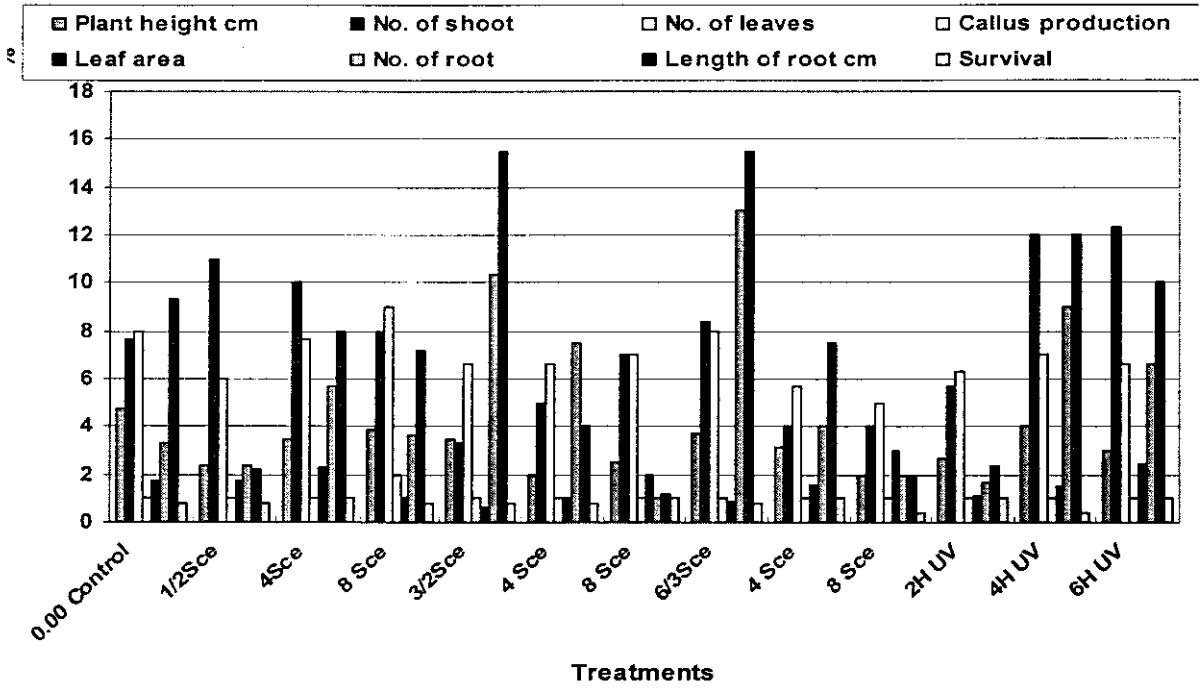

Fig. 2. Histogram shows the effect of irradiation for different periods by microwave and UV on growth of Yucca in the second culture.

with control (8.00) but the other treatments caused reduction .The power 1 for 8 seconds increased callus production 2.00 in comparison with control (1.00) Leaf area was 2.26, 2.00, 3.00, 2.46 cm^2 respectively for microwave power 1, 3, 6 and 6 hours UV respectively as compared with control (1.76 cm^2). The number of roots significantly increased; it was 5.66, 10.33, 13.00 and 9.00 for doses 1, 3, 6 microwave power and 6 hours UV respectively in comparison with the control (3.33cm). Also the root length was significantly increased; it was 15.50 cm, 15.50 cm and 12 cm in dose 3, 6 microwave power and 4 hours UV in comparison with the control (9.33cm). However the use of combined treatments was suppressing. Survival was significantly decreased to 40% by microwave power 6 for 8 second and 4 hours UV irradiation. Similar results were reported by Shibata and Kawata (1986); Shibata *et al* (1998); Nagatomi *et al* (1998); Youssef (2003) and Sayed *et al* (2005).

Effect of irradiation treatments on chlorophyll A and B and carotenoids

Data presented in Table (3) and (Fig. 3) showed the effect of different doses of microwave and ultraviolet treatments on chlorophyll- A and B and carotenoid content of Yucca. Data showed that control regenerated plants the lowest content of chlorophyll-A and B and carotenoids as compared with those treated with microwave and UV. On the other hand, chlorophyll- A and B and carotenoids decreased in the highest doses of microwave. Significant increases were observed when microwave were used at powers 1 and 3 for 8 seconds to 0.226 mg/g chlorophyll-A, 0.088 mg/g chlorophyll-B and 0.180 mg/g carotenoids in power 1 for 8 seconds, 0.122 mg/g chlorophyll-A, 0.047 mg/g chlorophyll-B and 0.094 mg/g Carotenoids in power 3 for 8 seconds in comparison with control (0.039 mg/g, 0.020mg/g and 0.034mg/g chlorophyll- A,B and Carotenoids respectively).

It is clear that control regerated plants showed a significant decrease in chlorophyll-A and B and carotenoids as compared with those irradiated by microwave power 6 for 8 seconds and reached to 0.024 mg/g chlorophyll-A, 0.013 mg/g chlorophyll- B and 0.021 mg/g carotenoids in comparison with control 0.039 mg/g, 0.020 mg/g and 0.034 mg/g chlorophyll- A, B and carotenoids, respectively.

On the other hand, ultraviolet treatment for 4 hours showed significant increase in these traits; it was 0.146 mg/g chlorophyll-A, 0.062 mg/g chlorophyll-B and 0.120 mg/g carotenoids in comparison with control 0.039 mg/g, 0.020mg/g and 0.034mg/g chlorophyll- A and B and carotenoids respectively. These results indicated the importance of microwave and ultraviolet rays treatments in inducing variabilities and agreed with those reported by (Nagatomi *et al* (1998)); Youssef (2003)and Sayed *et al* (2005).

Table 3. Effect of different doses of microwave and UV on chlorophyll A, B and carotenoids of Yucca.

Treatment doses in (watt) exposure	Exposure time	Chlorophyll-A mg/g	Chlorophyll-B mg/g	Carotenoids mg/g
Microwave				
control	0.00	0.039	0.021	0.034
1	2 Sec	0.089	0.031	0.074
1	4 Sec	0.081	0.028	0.052
1`	8 Sec	0.226	0.088	0.180
3	2 Sec	0.026	0.012	0.024
3	4 Sec	0.047	0.022	0.039
3	8 Sec	0.122	0.047	0.094
6	2 Sec	0.014	0.009	0.011
6	4 Sec	0.015	0.013	0.016
6	8 Sec	0.024	0.013	0.021
U.V				
80	2 hr	0.023	0.009	0.018
80	4 hr	0.146	0.062	0.120
80	8 hr	0.059	0.024	0.052
$LSD_{0.5}$		0.002	0.029	0.001

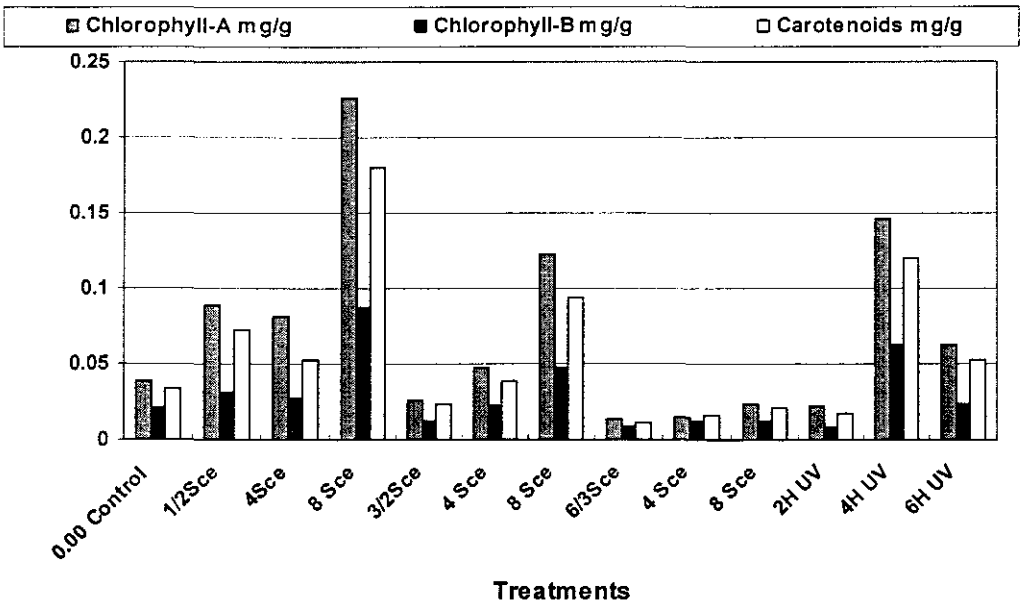

Fig 3. Histogram showing the effect of different doses of microwave and UV on Chlorophyll A, B and carotenoids of yucca.

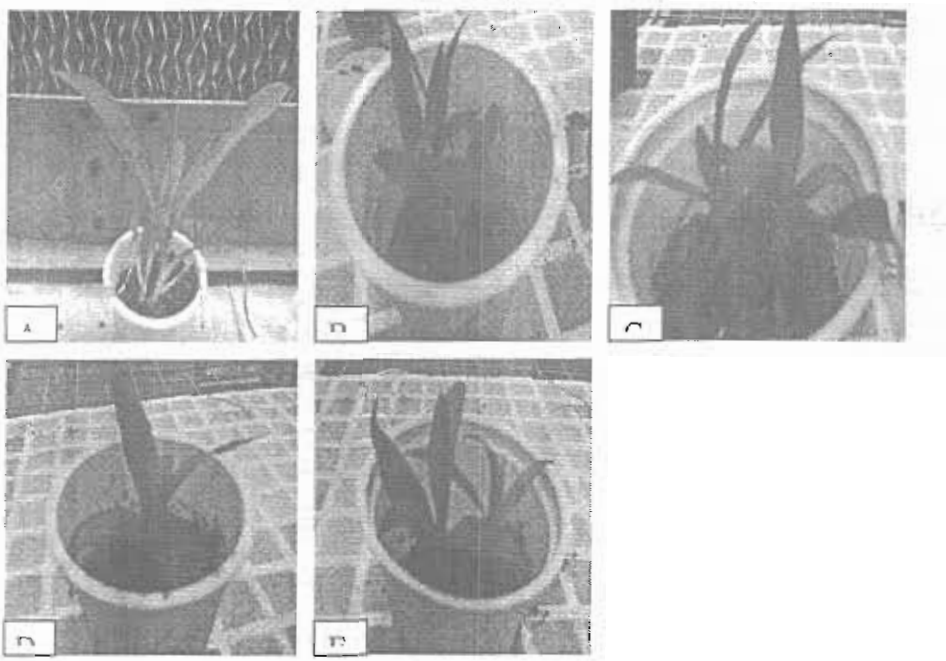

Photo 2. Acclimatized Yucca plantlets resulted from A=Con, B= 2 Sec, C=4 Sec, D=8 Sec, and UV ray E= 2 hr. (according to Youssef 2003).

Biochemical studies.

Gel electrophoresis technique has proved to be a useful tool for species identification. In the present study two isozymes i.e.poly phenyl Oxidase (PPO) isozymes, Peroxides (PRX), were assayed from leaves of yucca (Fig 5A and 6 B) showed the number of bands varied for the different irradiation with microwave and ultraviolet rays.The electrophoretic band pattern of Polyphenyl Oxidase (PPO) isozymes from leaves of yucca shown in (Fig. 6A). A total of 3 bands of Polyphenyl Oxidase isozymes were detected. The number of bands varied for the different irradiated materises clear a differences in (PPO) isozyme band pattern was detected in control 2 bands (track 1), while in irradiation with (power 3 for 8 seconds), 3 bands (track 2), and (power 6 for 8 seconds microwave)3 bands (track 3). On the other hand 3 bands (track 4,5) in irradiation with UV ray (4hr and6 hr) (Fig .6A).

Figure (5B) showed the electrophoretic pattern of peroxidase (PRX) isozymes in the yucca leaves explant and their regenerated plants growing on different medium types. A total of 4 peroxidase bands were detected The number of bands varied for the different Eradiated a clear differences in

(PRX) isozyme band pattern was detected in control 3 bands (track 1), and in eradiated with (3dose period 8 seconds) 3 bands (track 2),while in eradiated (6dose period 8 seconds microwave) 4 bands (track 3). On the other hand 3 bands (track 4, 5) in eradiated with Uv ray (4hr and6).

The observed different isozyme gene expiration specificity of isozymes has been demonstrated in several plant species Chawla (1988) Genetic changes which might indicate the occurrence of some mutation during *in vitro* cultures such as mitotic recombination, deletion, duplication, base changes or transposition have been considered as a possible cause behind the loss or appearance of some isozymes band in plant callus cultures (Arnison and Boll 1974).

Finally from the results presented it is clear that both UV and microwave treatment could be used successfully for mutation induction in yucca as a means of providing more variation in this asexually propagated explant to enrich variability and to select more useful variants.

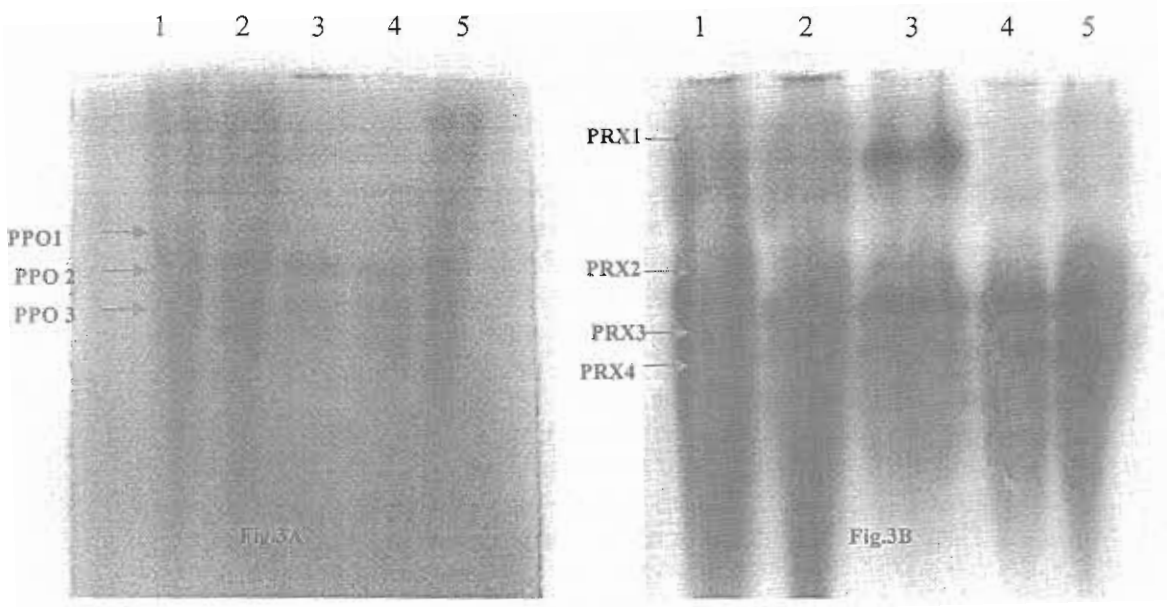

Photo 3A. Polyphenyl Oxidase isozymes banding pattern of yucca explant and their regenerated plant. Track 1(control), Track 2 (3dose period 8 seconds), track3 (6dose period 8 seconds microwave) and UV ray track4 (4hr), track 5 (6 hr).

Photo 3B. Peroxidase isozymes banding pattern of yucca explant and their regenerated plant. Track 1(control), Track 2 (3dose period 8 seconds), track3 (6dose period 8 seconds microwave) and UV ray track 4 (4hr), track 5 (6 hr).

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تاثيرالمعاملة بالأشعة الفوق بنفسجية والميكروويف علي نمو نبا ت اليوكا في المعمل

حنان محمد أحمد يوسف أحمد عثمان حمادة ريان

معهد بحوث البساتين ــ مركزالبحوث الزراعية ــ الجيزة ــ مصر

أجريت هذة التجربة بمعمل زراعه الأنسجة- معهد بحوث البساتين-مركز البحوث الزراعيــة بهدف إحداث بعض التغيرات او التباينات الوراثية الجسمية من خلال زراعة الأنسجة وذلك باستخدام كل من أشعة الميكروويف بجرعات ٠ ،٦،٣،١، "٠، ٩٥، ١٩٥، ٣٩٠" وات لمــدة ٢،٤،٨ ثــوانى وعلى قوى ٨٠ وات من الاشعة الفوق بنفسجية لمدة ٢،٤،٦ساعا ت لآســتخداث بعــض الطفــرات والتباينات. تم زراعة النبتات المعاملة على بيئة موراشيج واسكوج مضاف اليها ٦ بنزايل أمينوبيورين بتركيز ٢،٦ ملجم/لتر والاندول اسيتك أسيد بتركيز٢٥ .١ ملجم/للتر. ووجد أن المعاملة بــالميكروويف اكثر تاثير من الاشعة الفوق بنفسجية فى أحداث التباينات وأن الصفات الخضرية للنباتات قــد تــأثرت بالاشعةالمستخدمة حيث وجد أنة فى النقلة الاولى . أن المعاملة بقوى ٦ لمدة ٨ ثوانى أدت لزيادة طول النبتات وعدد الافرع معنويا وجميع المعاملات أدت لزيادة عددالاوراق بينما المعاملة بقوى ٣ لمــدة ٢ ثانية ادت لزيادة انتاج الكالس .كذلك وجد الاتى فى النقلة الثانية جميع المعــاملات أدت لتقليــل طــول النبات بينما المعاملة بقوى ١ لجميع الفترات والاشعة الفوق بنفسجية لمدة ٤ ,٦ ساعات أدت لزيــادة عدد الافرع . وكذلك أدت المعاملة بقوى ١ لمدة ٨ ثواني لزيادة عدد الاوراق والكــالس بينمــا زادت مساحة الورقة بالمعاملة بكل من قوى ١ لمدة ٤ ثوانى ٣ ،٦ لمدة ٨ ثوانى وكذلك بالمعاملة بالاشعة فوق بنفسجية لمدة ٤ ،٦ ساعات . أيضا وجد أن تعريض النبتات لاشعة الميكروويف لقوى ١ ،٣ لمدة ٨ ثوانى ادى لتحسين كل من كلورفيل أ ، ب والكاروتين مقارنة بالكنترول.

كذلك أظهرت هذه الأشعة المطفرة اختلافات على المستوى الانزيمى، لانزيمى البولى فنيل اوكسيديزوالبيروكسيديز. فعلى سبيل المثال أظهر التفريد الكهربائى لانزيم البولى فنيل اوكسيديز ٣ حزم عند تعريض النباتات لأشعة الميكروويف بقوى ٣ ،٦ لمدة ٨ ثوانى وللأشعة الفوق بنفسجية مقارنة

بالكنترول٢حزمة فى حين اظهر التفريد الكهربى لانزيم البيروكسيديز ٤ حزمة عند تعريض النباتات لأشعة الميكروويف بقوى٦ لمدة ٨ ثوانى مقارنة بالكنترول ٣ حزم و ٣ حزم عند تعريض النباتات لأشعة الميكروويف لقوي٣ لمدة ٨ ثوانى وكذلك للأشعة الفوق بنفسجية لمدة ٤و٦ ساعات مقارنة بالكنترول.