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SOME PHYSIOLOGICAL STUDIES ON INCREASING WATER STRESS TOLERANT OF MICROPROPAGATED SUGAR-CANE PLANTS BY USING LASER IRRADIATION.

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ABSTRACT

The aim of the present work was to study the effectiveness of Argon-laser irradiation (0.0, 40.0, 80.0, 120.0 and 160.0 minutes) in improving drought tolerance of the micropropagated sugarcane plants grown under different levels of PEG (0.0 4.0, 8.0, 12.0 and 16.0 %). For this reason, experiments were conducted during 2001 and 2002 seasons. The obtained results clearly confirmed the absolute superiority of lower dose (40 min.) of laser irradiation treatment, which significantly increases in the growth parameters of sugarcane grown *in vitro* (survival percentage, shootlet length, No. of leaves / shootlet. No. of roots / plantlet and fresh as well as dry weight of shootlet) and *ex vitro* (culm length , culm diameter, No, of leaves / plant and shoot dry weight) , yield components (cane and sugar yield / plant) and juice quality characters (percentage of sucrose, purity, sugar recovery and total soluble solids) over the untreated control treatment. Also, the sugarcane plantlets derived from shoot tip treated with the lower dose (40 min.) of laser rays were tolerant up to 8 % PEG level and were able to continue their growth under glasshouse conditions till maturity and cane production. The data also revealed that tolerance which was more pronounced as a result of the lower rate of laser rays and was associated with high accumulation of much more quantities inorganic osmotica,i.e. N, P , K, Mg and Ca as well as lowest quantities of Na, in addition to considerable accumulation of organic protective osmolytes (sucrose, proline, amino acids and total soluble phenols), photosynthetic pigments (carotenoids and chlorophylls) and endogenous hormones (IAA, GA_3 and ABA) in the sugarcane grown *in vitro* and *ex vitro* . Such accumulation increased as the PEG level was increased. Such behavior seems to induce more ability for sugarcane plants to continue their growth till maturity and production of cane even under 8 % PEG level. The obtained data suggested the possibility of successful application of the Argon-laser rays to improve drought tolerance of economic crops such as sugarcane.

INTRODUCTION

Sugarcane (*Saccharum officinarum L.*) is the most important sucrose-producing crop in Egypt. As the sugar is the most valued nutritional product, it is considered as an important source of energy required for human. The amount needed for the local consumption is greater than the total productivity from this crop in Egypt. Also. In the Arab World up till the year 1999, sugar gab reached 4.3 million ton that equal 1.323 Millar American Dollar representing 11.31 % of the total value of the main food gab (Allam, 2001 and El-Kholi, 2003). Thus, increasing its productivity and the cultivated areas are highly demanded in Egypt and the Arab World.

Most places in the world are subjected to environment stresses (salinity or drought). The Nile delta is widely known for its salt affected soils. Besides, vast areas of the Egyptian land are the arid zone where acute shortage of water supplies poses serious problems to plant growth and development. Drought is a major limiting factor in the production of field crops in the arid and semi-arid regions (Shin et al, 2000). Moreover, drought involved all conditions of water deficits below the requirements for normal plant growth (Anderson *et al.*, 1996). Drought resistance is therefore, the ability of a plant to resist all adverse conditions created by unfavorable phenomena (Pessaraki, 1994).

Water requirements for the growth of sugarcane plants are relatively quite high. Therefore, the limited share of the Nile water given to Egypt necessitates searching for ways and means of maximizing the efficiency of water usages by cutting down in water requirements in field crops and particularly sugarcane and rice. One effective approach is to develop sugarcane varieties that tolerate drought and salinity stress.

Biotechnology and plant tissue culture technique are effective tools for producing drought tolerant cell lines, tissues and plants. The micropropagation of shoot tip in vitro is the most common application of biotechnology in agriculture (Dole, 1990) . Also, it has been proposed on a useful, quick and economical to evaluate stress as well as a better system for testing and selecting for stress tolerance (Jose *et al.*, 2000 , Shin *et al.*, 2000 and Abd-Eltawab, 2001) .

Polyethylene glycol (PEG) is an inter, nonionic, long chain polymer ($HOCH_2$ - (CH_2 - O- CH_2) - CH_2 O), has been widely used to maintain experimental media at constant water potential values (Allen et al, 1981). Moreover, Dix (1993) mentioned that in vitro selected plantlet resistant to PEG induced water stress was described as somatically adapted in various species. It lowers the water potential but does not enter the cell wall, thus imposing a stress similar to that by desiccation (Sala *et al.*, 1990).

Irradiation with fast neutrons, laser and gamma rays may provide insight into the mechanism of action of the radiation in physiological and genetic variability, thus have been directly used to produce useful variation in quantitatively inherited characters, such as quality and maturity time (Cholakov, 1995). The induced gene mutations might involve the polygene of the qualitative traits, or the major genes controlling the qualitative (Ahmed, 1998). Therefore, the results previously obtained by several authors suggested the possibility of successful applications of gamma rays (Whan *et al.*, 1991 and b and Ghallab and Nesiem, 1999) and fast neutrons (El-Shafey *et al.*, 1994) to improve salinity tolerance of the sensitive wheat and rice cultivars, since, these mutagen agents are considered the most effective tools for inducing the useful genetical changes in the treated plant materials.

Therefore, the present work was conducted to study the effectiveness of laser rays treatment in improving drought tolerance of the microporpagated sugarcane as well as the produced plants grown under different levels of water stress, aiming to induce (*in vitro*) drought tolerant cell lines, tissues, plantlets and finally plants.

MATERIALS AND METHODS

The present work was carried out in the Plant Physiology Division, Faculty of Agric., Cairo Univ., as well as the laboratory of plant tissue culture, Sugar Crops Research Institute, Agricultural Research Center (A.R.C), Giza, during two successive years (2001-2002). Sugarcane cv. GT 54/9 was supplied from Sugar Crops Res., Inst., A.R.C, Giza, Egypt.

Preparation of the material and isolation of meristem

Actively growing shoots of sugar cane plants (cv. GT 54/9) are collected from (6-9) months old crop or from traditional cuttings grown in the field. Shoot tips with the growing apices were taken and the meristematic apex together with a base of tissue approx. 0.5 cm × 0.2 cm was cultured. Outer sheaths are removed by wiping the sheath with rectified spirit. The shoots are then washed with soap water for about 2-3 minutes followed by several changes of water for assuring the removal of most external contamination. The shoot tip explants was then thoroughly rinsed in 7o % ethyl alcohol (Ethanol) for 1 minute. Rinse with sterile distilled water 4-5 times till alcohol is completely washed off. Then the shoot tips immersed for 15 minutes in sodium hypochlorite (1.7-3.4%) or calcium hypochlorite (10%) and few drops of tween 20 were added as a wetting agent, then rinsed 10 minutes in 3 times in sterilized distilled water to remove all traces of the disinfectant. All steps of sterilization have been done under aseptic conditions inside the culture cabinet (laminar air flow) and by using sterilized instruments.

Established Shoot tip explants :

The following experiment was conducted with Murashige and Skoog (MS) basal medium (1962) .The pH of the prepared medium was adjusted at 5.7±0.1 prior to addition of agar at 7 g l^{-1}. The medium was distributed into the culture jars (325 ml) where each jar contained 50 ml of the medium. The jars were immediately capped with polypropylene closer, and then were autoclaved at 121°C at 15 lbs/inch for 20 min. The MS basal medium supplemented with 0.5 mg l^{-1} NAA +1mgl^{1} BAP + 30 g l^{-1} sucrose + 7 g l^{-1} agar was used as initiation medium for culturing sterilized shoot tip explants. The medium was distributed into culture jars (150 ml) where each jar contained 30 ml of medium. Shoot tip explants were incubated at day and night temperature of 27± 2°C. Light was provided by fluorescent lamps giving intensity of 1500 Lux for 16 hours per day.

Laser rays treatments:

Established shoot tip explants were transferred and cultured individually on shoot multiplication MS basal medium supplemented with100 mg l^{-1} myo-inositol + 30 g l^{-1} sucrose + 0.5 mg l^{1} nicotinic acid + 0.5 mg l^{1} pyrodoxine + 2 mg l^{1} glycine + 1.0 mg l^{1} NAA + 4.0 mg l^{1} BAA + 7 g l^{-1} agar. The explants were repeatedly subculture 3 times at 4 weeks intervals till obtaining cluster explants, each one containing 2-4 developed buds. Cluster

explants were laser irradiated (Argon) at doses of 0.0, 40.0, 80.0, 120.0 and 160.0 minutes. The laser irradiation (Argon) **from** " Argon Laser beam at 640 nm wavelength and 23 mwt. Output power. Type: 5500 A., USA " provided by the National Institute of Laser Enhanced Sciences, Cairo Univ., Giza , Egypt.

Polyethylenglycol (PEG) treatments :

Irradiated and un-irradiated explants were then transferred and cultured on multiplication basal MS medium supplemented with100 mg l^{-1} myo-inositol + 30 g l^{-1} sucrose + 0.5 mg l^{1} nicotinic acid + 0.5 mg l^{1} pyrodoxine + 2 mg l^{1} glycine + 1.0 mg l^{1} NAA + 4.0 mg l^{1} BAA + 6 g l^{-1} agar. Subculturing was done 7 times at 4 weeks intervals into corresponding multiplication fresh media.

Adventious growing shoots were then separated *in vitro* and transferred to rooting ½ MS basal medium supplemented with 2.0 mg l^{-1} NAA + 60 g l^{-1} sucrose + 3.0 g l^{1} activated charcoal + 6 g l^{-1} agar. Rooted plantlets were then transferred to MS basal liquid medium supplemented with 2.0 mg l^{-1} NAA + 60 g l^{-1} sucrose + 3.0 g activated charcoal. PEG (MW 8000) was added at the levels of 0.0, 4.0, 8.0, 12.0 and 16.0 % .Filter papers (Whatman, 90 mm) were designed in (M) letter shape as a bridge and inserted into the jars to be in contact with the medium in order to support plantlets. Osmotical potential (O.P.) of the medium was measured before culture and 4 weeks after culturing, using the electric osmometer (whereas 1000 mhos mol = 2.24 MPa). In all *in vitro* experiments each treatment consists of 20 replicates, each replicate consists of 12 jars where each jar contain one plantlet.

For each treatment 10 replicates, the morphological parameters (survival percentage, shoot height, leaves and roots number per plantlet, fresh and dry weights of the shoots) were recorded. The shootlets were dried in an oven at 70 °C for 48 hours and then crude dry weight were determined. The dried shootlets of each treatment were powdered and prepared for chemical analysis at the end of this period (6- weeks after culturing on rooting media). For proline, total soluble phenols, sugars, total free amino acids, endogenous phytohormones and photosynthetic pigments estimation, part of the shootlet was kept fresh

Acclimatization of the growing plants and cane production:

The produced plantlets were washed with tap water three times to remove all traces of agar; then immersed in vitafax (0.1% for 3 min.) and cultured individually in plastic pots (8 cm) containing a mixture of peatmoss and sand 1:1 (w/w); covered with white transparent plastic sheets (which were punched up 3 cm from two sides) under glasshouse conditions,i.e. light intensity of about 1500 Lux for 16 hours per day provided by white fluorescent lamps, the temperature of about 28±2°C and the relative humidity was adjusted to 85-90% by adding water for half hour every three hours through the mist during the nursery stage (21-30 days after transplanting). The white transparent plastic sheets were completely removed at the end of this stage. After two months, the acclimatized plants were transplanted to plastic pots, 40 cm. diameter, containing a mixture of clay and sand at a ratio

of 2:1 by weight (Ghallab and Nesiem, 1999). The chemical analysis of the used soil was carried out as described by Page *et al.*, (1982) were as follows :
PH = 7.63, EC = 0.69 ds/m, $HCO_3 + CO_3$ = 2.3 me/L , Cl^- = 10.9 me/L , SO_4 = 45.8 me/L , Ca = 7.9 me/L , Mg = 4,2 me/L , Na = 9,6 me/L an K = 2.4 me/L. The complete nutrient solution as described by Hewitt (1952) was used. The plants were irrigated at three days intervals with nutrient solution either alone for the control or mixed with the same previously mentioned levels of PEG concentrations throughout the whole growth stages. The plants were incubated at the glasshouse under the same experimental conditions for 8 months. In all pot experiments, each treatment consists of 10 replicates with 15 plants for each replicate (one plant for pot). During the growth period, one sample of 5 replicates; 15 plants from each treatment was taken at 90 days old. Culm length (cm), number of leaves / plant, culm diameter (cm) and dry weight of shoot were recorded. The shoots were dried in an oven at 70 °C for 48 hours and then the crude dry weight were determined. The dried shoots of each treatment were powdered and prepared for chemical analysis. For proline, sugars, total soluble phenols, total free amino acids, endogenous phytohormones and photosynthetic pigments estimation, part of the shoot system was kept fresh, and 5 replicates from each treatments were left to grow till harvest.
At harvesting stage, after 8 months in pots, cane and sugar yield / plant and juice quality characters were recorded. Sugar yield / plant was estimated according to the following equation: Sugar yield = cane yield / plant X sugar recovery %.
It is worth to be mentioned that all experiments in the two successive seasons were repeated 4 times

Statistical analysis:

Data of morphological characters of sugarcane grown *in vitro* and *ex vitro* as well as yield components and juice quality characters of sugarcane grown in *ex vitro* were statistically analyzed and the mean values were compared using L.S.D. values at 5 % levels (Gomez and Gomez, 1984).

Chemical analysis:

Nornai (1982) method was employed to determine the photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids).
The ethanol extracts of shoots were used to determinate reducing, non-reducing and total sugars, total free amino acids and total soluble phenols. Reducing, non-reducing and total sugars were determined by using phosphomolybdic acid reagent as described in **A.O.A.C. (1975).** Total free amino acids were determined by using ninhydrin reagent according to **Moore and Stein (1954).**
Free proline concentration was measured calorimetically in the extraction fresh shoots using ninhydrin reagent according to **Bates *et al* .(1973)**

The colorimetric method of Folin-Denis as described by **Swain and Hillis (1959)** was employed for determination the total soluble phenols.

The determination of N, P, K, Ca, Na, and Mg were carried out on the shoots ground dry material. Dry sample was digested by using sulphoric and perchloric acids according to **Piper (1947)**. Nitrogen was determined using the micro kejeldahl apparatus of Parnas-Wagner as described by Jones *et al.* (1991). Phosphorus was estimated calorimetrically by using chlorostannous reduced molybdophosphoric, blue color method according to Jackson **(1973)**. Sodium, magnesium, potassium and calcium were determined by using atomic absorption spectrophotometer (GBC,932 AA).
Extraction of plant hormones was carried out according to Sadeghian (1971). Methanolic extract of the fresh leaves were used for endogenous hormones estimation by Gas-liquied chromatography (GLC) [Ati-Unicam-610 Series) according to the method described by Vogel (1975). The glass Column (1.5 X 4 mm) was packed with 1% OV-17. Temperature: Injector 260°C, detector 300 °C and column initially for 3 min. at 200 °C then increased to 220 °C (rate 20 °C /min.) for 4 min., then increased again to 240 °C (rate 20 °C/min.) flow rates; carrier gas (N_2 special) 30 ml / min., hydrogen special 33 ml/min. and synthetic air 330 ml/min. and the chart speed 1 cm/min.

Juice quality:

At harvest, a sample of 15 stalks of sugarcane plants were chosen at random from each treatment. The primary juice was extracted by electric pilotmill (Sabri, 1966), screened and mixed thoroughly. One-liter juice was taken in glass cylinder to determine juice quality characters according to the formula as described by methods of Sugar and Integrated Industries Company (Anonymous, 1981).

1- Sucrose percentage was determined using sacharemeter according to A.O.A.C. (1975).
2- Purity percentage was calculated according to the following equation:

$$\textbf{Purity\%} = \frac{\textbf{Sucrose \%}}{\textbf{Brix \%}} \times 100$$

3- Sugar recovery percentage was calculated according to the following equation:

Sugar recovery % = Richness % X Purity %.

Where Richness = (Sucrose in 100 grams juice X Richness factor) / 100.

Richness Factor = 100 – [(Fiber % X physical impurities %) + water free from sugar %].

Where, Physical impurities % = 2.5, Water free from sugar % = 1.3.

4- Total soluble solids percentage [T.S.S. %] was determined by hand refractometer.

RESULT AND DISCUSSION

Osmotical potential:

PEG (for water stress) is an inert, non-ionic, large chain polymer (HO CH_2-(CH_2-O-CH_2) $_x$ -CH_2O) has been widely used to maintain experimental media

at predetermined ψw values (**Allan *et al.*, 1981**). It lowers the water potential but does not enter the cell wall thus imposing a stress similar to that by desiccation (**Sala *et al.*, 1990**). Data in Table (1) reveal that the gradual increase in PEG level resulted in gradual increase in the osmotical potential of the liquid media either before or after culture (Table, 1). Similarly, **Naidu *et al.* (1987)** observed significant reduction in leaf water potential (ψw), osmotic potential (ψs), turgor potential (ψp) and relative water content (R.W.C.) in response to water stress treatments, however, the osmotic pressure values reflects the water status in potential (ψp) and relative water content (R.W.C.) in response to water stress treatments, however, the osmotic pressure values reflects the water status in plant (**Batanony *et al.*, 1991**). The decrease in osmotic potential resulting from accumulation of solutes is a pronounced response to water stress in many plants. This process is known as osmotic adjustment; by lowering the osmotic potential in plants tissues subjected to water stress conditions (**Shin *et al.*, 2000**). Meanwhile, the increase in solute concentration is expected as a result of dehydration and decreasing cell volume, osmotic adjustment refers specifically to a net increase in solute concentration due to metabolic process trigged by stress. Osmotic adjustment generated a more negative leaf water potential, thereby, helping to maintain water movement into the leaf, consequently, leaf turgor. Solutes accumulate slowly during osmotic adjustment are relatively small, less than 1.0 MPa (**William, 1995**).

Table (1): Effect of polyethyleneglycol (PEG) levels on osmotical potential (mosmol) of the liquid medium before and after culture of rooted sugarcane plantlets.

Osmotic potential / PEG. levels (%)	Before culture	After culture
0	194.55	125.85
4	240.15	155.70
8	285.11	241.09
12	369.35	351.65
16	474.21	575.15
L.S.D. 5%	19.75	28.15

Growth characters :

The results in Tables (2 and 3) generally indicated that all growth characters of both sugarcane plantlets grown *in vitro*; i.e. survival percentage, shootlet length, No. of leaves / shootlet, No. of roots / plantlet, fresh and dry weight of shootlet (g/shootlet) and sugarcane plants grown in *ex vitro*; i.e. culm length (m.), culm diameter (cm.), No. of leaves/plant and dry weight of shoot (g/plant) gradual and significant decreased as the PEG levels increased (Mean P). These results are in agreement with those previously reported by Srivastava *et al.* (1997); Robertson *et al.* (1999) and Wiedenfeld (2000) on sugarcane plants.

Concerning the effect of PEG on decreasing plant growth characters, Nieman *et al.* (1988) reported that, the decrease in survival percentage under stress-condition may be due to the energy spent to maintain turgor pressure at

the expense of growth or the decrease in the availability of water to plants (Gunes *et al.*, 1996). Moreover, the disturbance in water uptake results in severely reduction in plant growth and maintenance (Rengel *et al.* 1999). However, when the soil water potential drops below a critical value, a water deficit in leaves will develop, this will associated with the depression in plant growth rate expressed by plants maintenance (Taize and Zaiger, 1991).

Table 2: Effect of laser irradiation doses on growth characters of sugarcane plantlets grown under different levels of PEG for 6 weeks after culturing on rooting media (Combined analysis for two seasons).

Laser irradiation doses (minutes)	Growth characters										
	Survival percentage						Shootlet length (cm)					
	PEG levels (%)					Mean (T)	PEG levels (%)					Mean (T)
	0	4	8	12	16		0	4	8	12	16	
Control	100	81.78	79.46	72.92	31.16	73.06	7.19	6.18	6.01	5.25	4.25	5.78
40	98.92	96.74	93.98	66.40	24.55	76.12	8.39	7.99	7.51	4.21	3.16	6.25
80	84.66	76.22	71.76	60.77	20.92	62.87	5.91	5.39	4.75	3.86	2.95	4.57
120	66.39	57.68	55.12	45.91	15.45	48.11	5.75	4.65	4.18	3.64	2.56	4.16
160	48.41	39.78	35.36	31.62	11.44	33.32	5.06	4.11	3.59	3.29	2.25	3.66
Mean (P)	79.68	70.44	67.14	55.52	20.70		6.46	5.66	5.21	4.06	3.03	
L.S.D. at 5%	T = 2.87 P=3.45 T*P= 6.51						T = 0.44 P = 0.51 T*P= 1.14					
	No. of leaves / shootlet						No. of roots / plantlet					
Control	7.45	6.53	6.07	5.21	4.62	5.98	7.56	6.66	6.19	5.31	4.66	6.08
40	8.66	8.14	7.74	4.19	3.59	6.46	8.83	8.22	7.89	4.23	3.68	6.57
80	6.33	5.85	5.45	3.95	3.31	4.98	6.46	5.92	5.56	4.02	3.35	5.06
120	6.01	5.16	4.93	3.51	3.01	4.52	6.13	5.21	5.01	3.67	3.09	4.62
160	5.83	4.92	4.57	3.09	2.88	4.26	5.94	5.02	4.63	3.16	2.95	4.34
Mean (P)	6.86	6.12	5.75	3.99	3.48		6.98	6.21	5.86	4.08	3.55	
L. S. D. at 5%	T = 0.38 P=0.54 T*P= 1.01						T = 0.37 P=0.44 T*P= 0.83					
	Shootlet fresh weight (g)						Shootlet dry weight (g)					
Control	9.93	8.88	8.01	6.29	5.76	7.77	0.48	0.41	0.36	0.31	0.26	0.36
40	11.26	10.95	9.73	5.23	4.45	8.32	0.69	0.61	0.56	0.19	0.13	0.43
80	8.61	7.99	6.75	5.01	4.10	6.49	0.34	0.27	0.22	0.15	0.11	0.22
120	6.93	5.84	4.69	4.11	3.88	5.09	0.29	0.24	0.19	0.12	0.09	0.19
160	5.89	4.77	4.38	3.82	3.05	4.38	0.25	0.20	0.15	0.10	0.08	0.16
Mean (P)	8.52	7.69	6.71	4.89	4.25		0.41	0.35	0.30	0.17	0.13	-
L. S. D. at 5%	T = 0.51 P=0.69 T*P= 1.29						T = 0.046 P=0.065 T*P= 0.121					

Peroxidase isozymes are involved in the cell wall structure and expansion, it could be considered as a key factor in cell response and adaptation to water stress. In this respect, Sancho *et al.*, (1996) reported that biotic stress induces biochemical changes such as the activity of peroxidase as a group of enzymes affected by stress conditions. On the other hand, Ullah *et al.* (1993) and Adams *et al.* (2004) demonstrated that the reduction in shoots growth might be due to the decrease in transpiration and photosynthesis under stress conditions. The reduction in leaves and roots number might be due to the imbalances in phytohormone levels under stress condition which may affect the biosynthesis or the destruction of plant hormones, i.e., the increase ethylene concentration (Wilkinson, 1994) and ABA accumulation (Shakirova and Bezrukova, 1998) or the reduction in endogenous IAA levels (Dunlap and Binzel, 1996).

Table 3: Effect of laser irradiation doses on growth characters of sugarcane plants grown in glass house conditions under the same different levels of PEG for go-days old after acclimatization (Combined analysis for two seasons).

Laser irradiation doses (minutes)	Growth characters											
	Culm length (m.)						Culm diameter (cm.)					
	PEG levels (%)					Mean (T)	PEG levels (%)					Mean (T
	0	4	8	12	16		0	4	8	12	16	
Control	3.15	2.84	2.66	2.55	2.43	2.73	2.55	2.37	2.22	2.10	1.97	2.24
40	4.06	3.85	3.69	1.91	1.79	3.06	3.79	3.54	3.35	1.40	1.25	2.67
80	2.47	2.16	1.99	1.85	1.66	2.03	1.84	1.64	1.51	1.36	1.21	1.51
120	2.17	2.09	1.81	1.77	1.59	1.89	1.79	1.55	1.46	1.29	1.15	1.45
160	1.99	1.88	1.75	1.64	1.48	1.75	1.68	1.46	1.33	1.25	1.09	1.36
Mean (P)	2.77	2.56	2.38	1.94	1.79		2.33	2.11	1.97	1.48	1.33	
L.S.D. at 5%	T = 0.24 P= 0.33 T*P= 0.62						T = 0.22 P = 0.36 T*P= 0.68					
	No. of leaves / plant						Shoot dry weight / plant (g)					
Control	18.20	16.93	15.69	13.83	10.99	15.13	110.13	98.41	83.63	69.39	56.79	83.67
40	21.94	20.52	18.73	11.12	8.25	16.11	152.36	140.68	131.84	53.98	41.88	104.15
80	15.42	13.13	12.69	10.55	7.32	11.82	94.95	83.84	68.73	47.52	39.58	66.92
120	13.83	12.42	11.81	9.72	6.89	10.93	79.83	72.21	60.64	43.26	35.93	58.37
160	12.78	11.65	10.77	8.55	6.38	10.03	70.59	63.39	51.59	38.68	33.77	51.60
Mean (P)	16.43	14.93	13.94	10.75	7.97		97.37	87.71	73.29	50.57	41.59	
L. S. D. a 5%	T = 0.75 P=1.43 T*P= 2.68						T = 5.38 P=7.90 T*P= 14.75					

The retardant in plant growth may be also explained by the great portion of energy will be used for water stress tolerance rather than for growth and biomass production of the organism. In addition, high metabolic activity is necessary for transformation ions from the aerial parts to the roots (Cornillon and Palloix, 1995). ATP is an important regulator of cell metabolism and UDPG could often be limiting factor for cell wall synthesis and growth (Marschner, 1995). In this respect, (Pessaraki, 2002) indicated that water stress condition reduced the synthesis of uridine nucleotides or the production of uridine triphosphate (UTP) and uridine diphosphate glucose (UDPG).

When consider the mean value of all growth characters of sugarcane grown *in vitro* and *ex vitro* due to each treatment (Mean T) regardless of the PEG level (Tables 2 and 3), it could be noticed that as the dose of Argon- laser rays increased, all growth characters gradually and significantly decreased when compared with the control treatment (0.0 minute) except for the lower dose (40 minute) which induced an opposite trend. The percentage of increment in the dry weight of sugarcane grown *in vitro* was 19.4% and *ex vitro* was 24.5% induced by the lower dose of laser ray over the control treatment. This increase of all growth characters might be due to stimulative effects of laser irradiation on the growth and dry matter accumulation. In this respect, Cholakov and Uzunovw (1997) reported that the helium – neon laser irradiation of cucumber seeds increased the growth characters of cucumbers plants grown under water stress conditions. Moreover, Ghallab and Omar (1998) showed that wheat grains presowing irradiation with ultraviolet laser rays (100 shoot) significantly increased the growth characters of wheat

plants. Recently, Bayerly (2003) indicated that gamma irradiation at lower doses (10 and 20 GY) had stimulus effects on growth characters of banana grown *in vitro* and *ex vitro* under water stress conditions.
Comparing the effect of the interaction between laser rays treatments and PEG levels clearly reveal that, at 4 and 8% PEG levels, treatment with 40 minute laser rays produced significantly higher values of the studied growth parameters when compared with the control treatment, while at 12% and 16% PEG levels, a negative trend were elicited. The same lower dose (40 min.) of laser rays treatment recorded the highest considerable increases in the dry weight of sugarcane grown *in vitro* (55.6%) and *ex vitro* (57.7%) as compared with the control treatment at 8% PEG level. Also, at the higher doses (80, 120 and 160 min.) of laser rays under all PEG levels, all growth characters of sugarcane grown *in vitro* and *ex vitro* were not significantly changed as compared with the control treatment.
This clearly indicates that the highest doses of laser rays have an inhibitory effect on the growth characters of sugarcane grown *in vitro* and *ex vitro*. Hence, the clear superiority of the lower dose (40 min.) of laser rays in inducing the highest degree of PEG tolerance of the sugarcane plants grown in *ex vitro* could be clearly seen and which even surpassed over untreated control. In accord with the obtained results in the present work, De-Guzman *et al.* (1982) mentioned that lower doses of gamma irradiation at 10 krad markedly stimulate the growth and the cultures were vigorously at 2.5 Krad that showed more shoots formation and more leaf production. Also, Whan *et al.* (1991) successfully produced two salt-resistant citurs mutants using gamma rays and EMS. Moreover, Ghallab and Nessiem (1999) who concluded that the possibility of successful application of gamma rays to improve salinity tolerance of the sensitive wheat cultivars. Recently, Bayerly (2003) reported that the possibility of successful application of gamma irradiation at lower doses (10 and 20 GY) to improve PEG tolerance of banana grown *in vitro* and *ex vitro*. Moreover, Khodary (2004) suggested the possibility of successful application of lower dose (25 GY) of gamma rays in improving salt tolerance of lupine plants.

2: Chemical Composition:

a- Sugar, proline, total free amino acids and total soluble phenols:

Sugars (reducing, non-reducing and total), proline, total free amino acids and total soluble phenols concentrations in the sugarcane grown *in vitro* and *ex vitro* increased dramatically (Mean P) by increasing PEG levels (Table 4). As with growth characters of sugarcane grown in vitro and ex vitro (Tables 2 and 3), the Mean T of the lower dose (40 min.) of laser rays which recorded considerably increased the accumulation of the protective compounds, i.e. sugars, proline, total free amino acids and total soluble phenols over the respective Mean T of the control treatment; meanwhile all other irradiation treatments (80, 120 and 160 min.) caused noticeable reduction in the accumulation of the protective compounds when compared with the control. Comparing the effect of the interaction between irradiation treatments and PEG levels clearly reveal that, the lower dose of laser rays accumulated much more concentrations of the protective compounds in the

sugarcane grown *in vitro* and *ex vitro* which greatly exceeded control treatment up to 8% PEG.

On the contrary all other irradiation (80, 120 and 160 min.) treatments under all PEG levels and 12% as well as 16 % of PEG levels for lower dose of laser rays caused noticeable reduction in the accumulation of the protective compounds when compared with control. Hence, the regenerated sugarcane plants from cluster explants exposed to 40 minute of laser rays exhibited the highest degree of PEG tolerant, i.e. the positive correlation between such treatment and improving of PEG tolerance. In this respect, Carpita *et al.* (1990) explained the cell adaptation to PEG, stress by additional accumulation of sugars, amino acids and other metabolically protective osmolites. Also, the same authors suggested that starch and polysaccharides are converted to simple sugars to maintain more negative water potential values inside the plant, the sugars as osmolytes enable plants to keep better water relations under water stress conditions.

Furthermore, Wilkinson (1994) reported that under water stress and saline conditions, the accumulation of non toxic substances such as sucrose, proline, organic acids, pigments, nucleic acids and protein are considered to be protective adaptation and the survival of plants under water stress and saline conditions depends upon the regulation of metabolic processes and the quantitative ratio between the protective and toxic metabolic intermediate. Moreover, it has been suggested that the high concentration of organic solutions in the cytoplasm could have the following roles: a- a contribution to the osmotic balance when electrolytes are lower in the cytoplasm than the vacuole, b- a protective effect of enzymes in the presence of high electrolytes in the cytoplasm (Marschner, 1995).

The sugars as osmolytes can enable plants to keep better water relations under stress conditions. Also, sucrose protected isolated chloroplasts against desiccation (Rangel, 1999). More recently, Pessaraki (2002) concluded that plant use soluble sugars as an osmoticum under water stress and saline conditions. Hence, the plants that fail to increased soluble sugars biosynthesis could not tolerate salt stress. El-Shafey *et al.* (2003) reported that salt-tolerant Sakha 8 wheat cultivar showed much higher degree of osmotic adjustment through the accumulation of considerable quantities of organic protective osmolytes, i.e. sugars (especially non-reducing ones), proline and free amino acids in their shoots and roots, which greatly exceeded that in the salt susceptible to Giza 167 wheat cultivar. Moreover, Ragab and Radshed (2003) with grain sorghum and Bayerly (2003) with banana grown *in vitro* and *ex vitro* found that proline and sugars increased with increasing PEG Level.

Concerning accumulation of phenols and amino acids with increasing water stress levels, Hanafy Ahmed (1991) noticed that the increasing in sugars, total free amino acids and total soluble phenols concentrations when plants subjected to stress conditions could be explained on the assumption that such plants might have less efficiency to condensate simple organic compounds into more complex one. In addition, the same author assumed that the higher level of total soluble phenols and total free amino acids concentrations might be due to the increase in the metabolic activity to synthesis shikimic acid. Moreover, Anderson *et al.* (1995) mentioned that, the

accumulation of amino acids under drought stress condition was due to the synthesis of organic acids and not from the hydrolysis of proteins or from preexisting of amino acids. It has been suggested that organic acids (citric, malic....etc) served as a C source in the synthesis of amino acids, i.e. proline *via* glutamate dehydrogenase.

Table 4: Effect of laser irradiation doses on sugars (reducing, non-reducing and total) as mg glucose / g. F. W., proline, total free amino acids and total soluble phenols as mg/g F.W. of sugarcane grown *in vitro* for 6 weeks after culturing on rooting media and *ex vitro* for 90-days old after acclimatization (Combined analysis for two seasons).

Laser irradiation doses (minutes)	*In vitro* plants											
	Reducing sugars						Non-reducing sugars					
	PEG levels (%)					Mean (T)	PEG levels (%)					Mean (T)
	0	4	8	12	16		0	4	8	12	16	
0	3.28	4.18	5.17	7.15	7.25	5.41	12.35	15.71	16.65	20.48	21.03	17.24
40	5.01	6.11	6.67	6.81	6.96	6.31	16.67	18.22	19.15	19.64	19.99	18.73
80	3.01	3.88	4.75	5.99	6.04	4.73	11.16	14.29	15.31	18.64	19.11	15.70
120	2.83	3.61	4.35	5.21	5.52	4.30	10.01	13.43	14.25	17.33	18.58	14.72
160	2.57	3.35	4.02	4.49	4.88	3.86	9.44	12.76	12.96	15.25	16.34	13.35
Mean (P)	3.34	4.23	4.99	5.93	6.13		11.93	14.88	15.66	18.27	19.01	
	Total sugars						Total free amino acids					
0	15.63	19.89	21.82	27.63	28.28	22.65	3.54	4.11	5.19	6.72	6.94	5.30
40	21.68	24.33	25.82	26.45	26.95	25.04	4.98	6.61	6.37	6.55	6.73	6.13
80	14.17	18.17	20.06	24.63	25.15	20.43	3.22	3.74	4.75	6.15	6.26	4.82
120	12.84	17.04	18.60	22.54	24.10	19.02	2.99	3.47	4.41	5.61	5.94	4.48
160	12.01	16.11	16.98	19.74	21.22	17.21	2.81	3.27	4.26	5.34	5.52	4.24
Mean (P)	15.27	19.11	20.65	24.20	25.14		3.51	4.12	4.99	6.07	6.27	
	Proline						Total soluble phenols					
0	1.16	1.48	2.16	2.83	2.92	2.11	2.12	2.27	3.39	5.44	5.55	3.75
40	1.99	2.41	2.67	2.71	2.79	2.51	4.31	4.62	5.17	5.22	5.33	4.93
80	1.07	1.36	1.98	2.51	2.64	1.91	1.97	2.08	3.15	4.65	4.92	3.35
120	0.98	1.27	1.84	2.33	2.45	1.77	1.88	1.98	2.94	4.27	4.61	3.14
160	0.92	1.19	1.77	2.11	2.21	1.64	1.74	1.87	2.75	3.11	3.52	2.59
Mean (P)	1.22	1.54	2.08	2.52	2.58		2.40	2.56	3.48	4.54	4.79	
Acclimatized plants *(ex vitro)*												
	Reducing sugars						Non-reducing sugars					
0	3.67	4.72	5.84	8.07	8.19	6.09	13.79	16.65	17.84	21.71	22.29	18.46
40	5.61	6.91	7.54	7.69	7.86	7.12	17.65	19.31	20.31	20.82	21.19	19.86
80	3.42	4.38	5.37	6.77	6.83	5.35	11.83	15.15	16.23	19.76	20.26	16.65
120	3.19	4.07	4.92	5.89	6.24	4.86	10.61	14.24	15.11	18.37	19.69	15.60
160	2.90	3.79	4.54	5.07	5.51	4.36	10.02	13.53	13.74	16.17	17.31	14.15
Mean (P)	3.76	4.77	5.64	6.69	6.93		12.78	15.78	16.65	19.37	20.15	
	Total sugars						Total free amino acids					
0	17.46	21.37	23.68	29.78	30.48	24.55	4.11	4.77	6.02	7.79	8.05	6.15
40	23.26	26.22	27.85	28.51	29.05	26.98	5.78	6.97	7.39	7.59	7.81	7.11
80	15.25	19.53	21.60	26.53	27.09	22.00	3.74	4.34	5.51	7.13	7.26	5.59
120	13.80	18.31	20.03	24.26	25.93	20.46	3.47	4.03	5.12	6.51	6.89	5.20
160	12.92	17.32	18.28	21.24	22.82	18.51	3.26	3.79	4.94	6.19	6.40	4.92
Mean (P)	16.54	20.55	22.29	26.06	27.08		4.07	7.78	5.79	7.04	7.28	
	Proline						Total soluble phenols					
0	1.52	1.96	2.83	3.71	3.83	2.77	2.61	2.79	4.17	6.69	6.83	4.62
40	2.63	3.16	3.49	3.55	3.65	3.29	5.32	5.68	6.36	6.42	6.55	6.07
80	1.41	1.78	2.59	3.29	3.46	2.51	2.42	2.56	3.87	5.72	6.05	3.95
120	1.28	1.66	2.41	3.05	3.21	2.32	2.31	2.44	3.62	5.25	5.67	3.86
160	1.21	1.55	2.32	2.76	2.89	2.15	2.14	2.31	3.38	3.83	4.33	3.19
Mean (P)	1.61	2.02	2.73	3.27	3.41		2.96	3.16	4.28	5.58	5.89	

Recently, Bayerly (2003) with banana found phenols and amino acid increased with increasing PEG level. Also, Ragab and Rashed (2003) with grain sorghum, found that amino acids increased with increasing water stress level.

The endogenous concentration of free proline in plants can be used as an indicator of salt and water stress tolerance. For each plant, it appears that there is an external salt concentration above, which the plant's proline level sharply rises. This critical point is directly to the ability of plant to tolerante salt. Thus, measurements of condition can be used to determine salt resistance of plants (Pessarakli, 2002). Moreover, proline and other compatible solutes are believed to case the minimal inhibition of metabolism. Also, proline is organic osmolytes solute with an amphiphilic molecule protects the hydrophilic parts (Binzel and Reuveni, 1994). In addition, Good and Zaplachinski (1994) reported that, the concentration of free amino acids (particularly proline) often increases markedly in the leaves or other plant tissues with exposure to many biotic or abiotic stress. Recently, Salem *et al.* (2002) with faba bean, Fatouh Youssef (2003) with wheat and Khodary (2004) with lupine found that proline, sugars and free amino acids increased with increasing salinity level.

New class of genes, called "Osm" (Osmotic tolerance) genes that is used for protection against osmotic stress and may work in a similar manner in plants, bacteria and animals now attracted the attention of physiologists, through their action following salinity. The over produced proline may be explained on the basis that osmogenes govern the production of a class of molecules such as betaine and proline that protect the cell and its constituents against "dehydration Osm" (Pessarakli, 2002). Also, many reports proved the rapid increase in synthesis and accumulation of sugar under water stress and saline conditions. Nasir *et al.* (2000) reported that leaves of salt tolerant line sugarcane showed high degree of osmotic adjustment by the accumulation of more K^+, free proline and sugar contents. Cordoba *et al.* (2001) found that roots salt-treated of *Chloris gayana* plants accumulated higher concentrations of soluble sugars.

As for the efficiency of laser rays in this regard, Cholakov and Uzunov (1997) suggested the possibility of successful application of laser rays to improve water stress tolerance of cucumber plants, which was associated with increasing accumulation of the protective substances such as total sugars, free amino acids, proline and soluble phenols in the cucumber plants with increasing water stress levels. Moreover, Zheng et al (1991) and Ghallab and Omar (1998) found that irradiation of wheat grains with the low rate of ultraviolet laser rays (100 shoot) increased total sugars and free amino acids a swell as protein percentage in the wheat plants and grains.

b- Photosynthetic Pigments

Comparing the results obtained in Table 5, clearly revealed that photosynthetic Pigments concentration, i.e. chlorophylls (a, b and total) and carotenoids decreased in the sugarcane grown *in vitro* and *ex vitro* with increasing PEG level (Mean P). As with growth characters (Tables 2 and 3) and protective compounds (Table 4) of sugarcane grown *in vitro* and *ex vitro*, the Mean T of the lower rate (40 min.) of laser irradiation which recorded considerably increased the accumulation of the photosynthetic pigments over

the respective Mean T of the control treatment; meanwhile all other irradiation treatments (80, 120 and 160 min.) exhibited an opposite trend. Comparing the effect of the interaction between irradiation treatments and PEG levels clearly reveal that, the lower rate (40 min.) of laser irradiation accumulated much more concentrations of the photosynthetic pigments in the sugarcane grown *in vitro* and *ex vitro* which greatly exceeded control treatment up to 8% PEG level. On the contrary, all other irradiation (80, 120 and 160 min.) treatments under all PEG levels and 12% as well as 16% PEG levels for lower rate (40 min.) of laser irradiation caused noticeable reduction in the accumulation of the photosynthetic pigments when compared with control treatment.

Table 5: Effect of laser irradiation doses on photosynthetic pigments as mg / g. F. W., of sugarcane grown *in vitro* for 6 weeks after culturing on rooting media and *ex vitro* for 90-days old after acclimatization (Combined analysis for two seasons).

Laser irradiation doses (minuets)	*In vitro* plants											
	Chlorophyll a						Chlorophyll b					
	PEG levels (%)					Mean (T)	PEG levels (%)					Mean (T)
	0	4	8	12	16		0	4	8	12	16	
0	1.42	1.37	1.23	1.16	0.88	1.21	0.67	0.52	0.46	0.38	0.31	0.47
40	1.73	1.66	1.55	1.02	0.77	1.35	0.81	0.75	0.61	0.34	0.29	0.56
80	1.27	1.19	1.04	0.94	0.69	1.03	0.56	0.44	0.37	0.29	0.26	0.38
120	1.18	1.05	0.95	0.88	0.58	0.93	0.52	0.41	0.31	0.27	0.24	0.35
160	1.07	0.96	0.82	0.73	0.51	0.82	0.44	0.36	0.29	0.23	0.19	0.30
Mean (P)	1.33	1.25	1.12	0.95	0.69		0.60	0.49	0.41	0.30	0.26	
	Carotenoids						Total chlorophylls					
0	0.59	0.43	0.37	0.32	0.28	0.39	2.09	1.89	1.69	1.54	1.19	1.68
40	0.75	0.59	0.53	0.29	0.24	0.48	2.54	2.41	2.16	1.36	1.06	1.91
80	0.48	0.36	0.31	0.24	0.22	0.32	1.83	1.63	1.41	1.23	0.95	1.41
120	0.44	0.33	0.29	0.22	0.19	0.29	1.70	1.46	1.26	1.15	0.82	1.28
160	0.39	0.28	0.25	0.20	0.18	0.26	1.51	1.32	1.11	0.96	0.70	1.12
Mean (P)	0.53	0.39	0.35	0.25	0.22		1.93	1.74	1.53	1.25	0.95	
Acclimatized plants *(ex vitro)*												
	Chlorophyll a						Chlorophyll b					
0	1.82	1.75	1.57	1.48	1.13	1.55	0.84	0.61	0.52	0.47	0.39	0.57
40	2.21	2.12	1.98	1.31	0.99	1.72	1.02	0.95	0.87	0.41	0.35	0.73
80	1.63	1.53	1.33	1.20	0.88	1.31	0.71	0.55	0.47	0.37	0.33	0.49
120	1.51	1.34	1.22	1.13	0.74	1.19	0.66	0.52	0.39	0.34	0.30	0.44
160	1.37	1.23	1.05	0.93	0.65	1.05	0.55	0.45	0.35	0.29	0.23	0.37
Mean (P)	1.71	1.59	1.43	1.21	0.88		0.76	0.62	0.52	0.38	0.32	
	Carotenoids						Total Chlorophylls					
0	0.78	0.57	0.49	0.43	0.37	0.53	2.66	2.36	2.09	1.95	1.52	2.12
40	1.02	0.81	0.72	0.37	0.31	0.65	3.23	3.11	2.85	1.72	1.34	2.45
80	0.63	0.46	0.41	0.32	0.29	0.42	2.34	2.08	1.80	1.57	1.21	1.80
120	0.59	0.42	0.39	0.27	0.24	0.38	2.17	1.86	1.61	1.47	1.04	1.63
160	0.51	0.37	0.33	0.25	0.21	0.33	1.92	1.68	1.40	1.22	0.88	1.42
Mean (P)	0.71	0.53	0.47	0.33	0.28		2.47	2.21	1.95	1.59	1.20	

In this respect Pessaraki (2002) indicated that the depressive effect of water stress conditions on the absorption of some ions which was involved in the chloroplast formation such as Mg and Fe could be expected as reason for chlorophyll suppression in leaves, and / or increase in growth inhibitors such as ethylene or abscisic acid production which enhanced senescence under stress conditions. In addition, un-available uptake of specific ions by the plants, and the accumulation of some ions in the leaves are widely assumed to result in the

inhibition of photosynthesis. However, biosynthesis of chlorophyll and subsequently CO_2 fixation were inhibited under water stress conditions (Makino *et al.* 1997).
Moreover, Cholakov and Uzunovw (1997) found that irradiation of cucumber seeds with laser rays increased photosynthetic pigments concentration in the cucumber plants with increasing water stress level.

C- Minerals :

The obtained data in Table 6 clearly show that the concentrations of Na, Mg and Ca gradually increased in the sugarcane grown *in vitro* and *ex vitro* as PEG levels increased, the concentrations of N, P and K exhibited an opposite trend (Mean P). Also, the Mean T of the lower dose (40 min.) of laser rays recorded considerably increased the accumulation of nutrient elements concentrations over the respective Mean T of the control treatment; meanwhile all other irradiation treatments (80, 120 and 160 min.) exhibited an opposite trend. Comparing the nutrient elements concentrations of the treated plants shows that the accumulations of N, P, K, Na, Ca and Mg uptake into sugarcane grown *in vitro* and *ex vitro* treated with 40 min. of laser rays as the PEG level increased up to 8% PEG, while all irradiation treatments (80, 120 and 160 min.) under all PEG levels and 12% as well as 16% PEG levels for 40 min of laser rays treatment greatly decreased such uptake and accumulations when compared to the respective values of the untreated control treatment. This strongly emphasized the superiority of laser rays at lower dose (40 min.) in stimulating the uptake and accumulations of various nutrient elements as previously reported for sugars, proline, total free amino acids and total soluble phenols (Table 4), photosynthetic pigments (Table 5) and simulative effects on growth characters (Tables 2 and 3) of sugarcane grown *in vitro* and *ex vitro* under different levels of PEG up to 8% PEG level.
The favorable effects of the low dose of laser rays (40 min.) were reflected on the growth (Tables 2 and 3), protective compounds (Table 4) and Photosynthetic pigments (Table 5). These effects may be as a result of plant adaptation to stress conditions. Increasing nutrient accumulation induced by laser rays under water stress conditions was previously recorded by Cholakov and Uzunov (1997) with cucumber.

The reduction in N under water stress and saline conditions may be due to reduction in water absorbed and a decrease in root permeability (Pessaraki, 2002). Under stress conditions, Na influx across the plasmalemma to the vacuole may play a major role in permitting turgor maintenance. Some crops show marked beneficial effects of Na especially if the K supply is limiting. These crops take up large amount of Na^+ which contributes to the osmotic potentials of the leaves and increase resistance to water stress. The damage effect of Na, however, may be attributed to that Na is capable of disturbing the fine structure of plant cell causing swelling of chloroplast which may result in chlorosis and necrosis (Wilkinson, 1994).

Furthermore, Taiz and Zeiger (1991) postulated that Mg concentration in chloroplasts may influence photosynthesis during water stress through its role in coupling electron transport to ATP production. The plants with the lower tissue Mg concentrations maintained higher photosynthetic rates as leaves

became hydrated. Also, Marschner (1995) indicated that Ca^{++} is strongly competitive with Mg^{++} and binding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg^{++} than for Ca^{++}. Moreover, Trivedi et al, (1991) and El-Shafey *et al.* (2003) working on wheat callus and they found that total N, P, K and Ca decreased with increasing salinity level. Also, Samarah *et al.* (2004) working on soyabean, found that N, P and K concentrations decreased with increasing drough stress level.

Table 6: Effect of laser irradiation doses on nitrogen, phosphorus, potassium, calcium, sodium and magnesium concentrations (mg/g D.W.) of sugarcane grown *in vitro* for 6 weeks after culturing on rooting media and *ex vitro* for go-days old after acclimatization (Combined analysis for two seasons).

Laser irradiation doses (minutes)	*In vitro* plants											
	Nitrogen						Phosphorus					
	PEG levels (%)					Mean (T)	PEG levels (%)					Mean (T)
	0	4	8	12	16		0	4	8	12	16	
0	29.51	26.18	22.19	17.14	15.76	22.16	2.43	2.22	2.01	1.83	1.61	2.02
40	41.32	38.48	33.27	15.66	14.83	28.71	2.76	2.66	2.51	1.68	1.47	2.22
80	25.96	23.31	19.31	14.41	13.62	19.32	2.23	2.04	1.85	1.55	1.35	1.80
120	23.15	20.47	17.95	13.39	11.72	17.34	1.96	1.89	1.71	1.46	1.28	1.66
160	20.49	18.19	14.52	11.92	10.55	15.13	1.84	1.75	1.63	1.39	1.21	1.56
Mean (P)	28.09	25.33	21.14	14.50	13.29		2.24	2.11	1.94	1.58	1.38	
	Potassium						Calcium					
0	38.21	36.73	32.86	23.49	20.15	30.29	10.87	11.54	12.46	21.52	22.51	15.78
40	49.82	47.12	44.92	21.85	18.74	36.49	15.56	17.75	19.22	20.35	21.11	18.79
80	35.15	33.42	28.92	20.31	17.61	27.08	9.89	10.51	11.33	17.11	18.81	13.53
120	31.92	29.56	26.72	18.69	16.92	24.76	9.27	9.76	10.66	15.04	16.34	12.21
160	27.11	25.92	21.45	17.71	15.35	21.51	8.65	9.17	9.93	11.81	13.56	10.62
Mean (P)	36.44	34.55	30.97	20.41	17.75		10.85	11.75	12.72	17.17	18.47	
	Sodium						Magnesium					
0	1.65	1.83	2.01	2.21	2.59	2.06	2.22	2.63	3.01	4.39	4.51	3.35
40	1.01	1.25	1.46	2.35	2.73	1.76	3.49	3.75	3.98	4.02	4.23	3.89
80	1.77	1.96	2.19	2.49	2.92	2.27	2.01	2.39	2.73	3.69	3.81	2.93
120	1.91	2.07	2.32	2.65	3.05	2.40	1.92	2.21	2.51	3.21	3.55	2.68
160	2.11	2.29	2.49	2.79	3.22	2.58	1.84	2.09	2.29	2.93	3.11	2.45
Mean (P)	1.69	1.88	2.09	2.49	2.90		2.29	2.61	2.90	3.65	3.84	
Acclimatized plants *(ex vitro)*												
	Nitrogen						Phosphorus					
0	39.84	35.34	29.96	23.14	21.28	29.91	2.65	2.41	2.19	1.99	1.75	2.19
40	55.78	51.85	44.92	21.16	20.01	38.74	3.01	2.89	2.74	1.83	1.61	2.42
80	35.14	31.46	26.16	19.45	18.39	26.12	2.44	2.22	2.02	1.69	1.47	1.97
120	31.25	27.63	24.23	18.02	15.82	23.39	2.15	2.07	1.86	1.59	1.39	1.81
160	27.66	24.56	19.61	16.19	14.24	20.45	2.01	1.91	1.78	1.52	1.32	1.71
Mean (P)	37.93	34.17	28.98	19.59	17.95		2.45	2.30	2.12	1.72	1.51	
	Potassium						Calcium					
0	46.23	44.44	39.76	28.42	24.38	36.65	12.61	13.39	14.45	24.96	26.11	18.30
40	60.28	57.11	54.35	26.44	22.67	44.17	18.05	20.59	22.29	23.61	24.49	21.81
80	42.53	40.43	34.99	24.58	21.31	32.77	11.47	12.19	13.14	19.85	21.82	15.69
120	38.62	35.77	32.33	26.61	20.47	30.76	10.75	11.32	12.37	17.45	18.95	14.17
160	32.81	31.36	25.95	21.43	18.57	26.02	10.03	10.64	11.52	13.69	15.73	12.32
Mean (P)	44.09	41.82	37.48	25.49	21.48		12.58	13.63	14.75	19.91	21.42	
	Sodium						Magnesium					
0	2.13	2.36	2.59	2.85	3.34	2.65	3.39	2.84	3.25	4.74	4.87	3.82
40	1.30	1.61	1.88	3.03	3.52	2.27	3.77	4.05	4.29	4.34	4.57	4.20
80	2.28	2.53	2.83	3.21	3.77	2.92	2.19	2.58	2.95	3.99	4.11	3.16
120	2.46	2.67	2.99	3.42	3.93	3.09	2.08	2.39	2.72	3.47	3.83	2.89
160	2.72	2.95	3.21	3.59	4.15	3.32	1.99	2.26	2.47	3.16	3.36	2.65
Mean (P)	2.18	2.42	2.70	3.22	3.74		2.68	2.82	3.14	3.94	4.15	

D- Phytohormones:

The obtained data in Table 7 regarding hormonal analysis of the sugarcane grown *in vitro* and *ex vitro* clearly reveal that the concentrations of indole – 3 – acetic acid (IAA) and gibberelic acid (GA_3) (mg/g F. W.) were decreasing by increasing PEG levels (Mean P) to reach their lowest values at the highest level of PEG, i.e. 16% PEG level, meanwhile the concentrations of abscisic acid (ABA) contrary increased. Similar results were reported by Ragab and Rashad (2003) under water stress conditions and El-Antably *et al.* (1994), Amer *et al.* (1995), Ibrahim and Shehata (2000) and El-Shafey *et al.* (2003) under saline conditions.

Table 7: Effect of laser irradiation treatments on indole-3- acetic acid (IAA), gibberelic acid (GA_3) and abscisic acid (ABA) concentrations (µg/g F.W.) in the leaves of sugarcane grown *in vitro* for 6 weeks after culturing on rooting media and *ex vitro* for go-days old after acclimatization (Combined analysis for two seasons).

Laser irradiation doses (minutes)	*In vitro* plants											
	IAA						GA_3					
	PEG levels (%)					Mean (T)	PEG levels (%)					Mean (T)
	0	4	8	12	16		0	4	8	12	16	
0	19.02	15.47	13.66	11.65	10.98	14.16	20.99	18.98	16.69	14.49	11.73	16.58
40	30.12	27.66	22.97	10.99	9.84	20.32	28.69	25.48	23.35	12.66	8.39	19.71
80	16.69	13.35	12.44	9.67	7.65	11.96	15.36	13.85	11.65	9.33	6.54	11.35
120	15.78	11.87	9.91	7.33	6.46	10.27	12.76	11.39	10.81	8.21	5.95	9.82
160	13.88	9.91	7.48	6.17	5.43	8.57	11.55	9.85	8.56	7.78	5.41	8.63
Mean (P)	19.09	15.65	13.29	9.16	8.07		17.87	15.91	14.21	10.49	7.60	
	Acclimatized plants (*ex vitro*)											
	IAA						GA_3					
0	22.38	18.52	17.29	15.34	11.88	17.08	24.67	21.41	19.64	17.58	13.78	19.42
40	35.44	32.67	26.95	14.97	10.81	24.17	34.48	29.98	25.96	15.11	11.18	23.34
80	19.96	16.11	14.88	13.17	8.75	14.57	21.99	19.34	16.14	13.56	9.45	16.09
120	18.68	13.87	11.67	8.95	8.31	12.29	19.75	16.88	13.71	9.19	7.61	13.43
160	16.22	11.65	9.23	8.11	7.66	10.57	15.31	12.65	11.96	8.59	6.18	10.94
Mean (P)	22.54	18.56	16.00	12.11	9.48		23.24	20.05	17.48	12.81	9.64	
	ABA											
	In vitro plants						Acclimatized plants (*ex vitro*)					
0	2.04	2.46	2.62	3.36	3.49	2.79	2.43	3.04	3.19	4.10	4.21	3.39
40	2.91	3.03	3.18	3.24	3.36	3.14	2.65	3.69	3.83	3.99	4.11	3.65
80	1.82	2.09	2.31	2.85	2.97	2.41	2.14	2.89	3.05	3.71	3.92	3.14
120	1.66	1.84	2.14	2.59	2.66	2.18	2.05	2.71	2.91	3.59	3.74	3.00
160	1.53	1.67	2.01	2.31	2.52	2.01	1.91	2.66	2.83	3.42	3.61	2.89
Mean (P)	1.99	2.22	2.45	2.87	3.00		2.24	2.99	3.16	3.76	3.92	

Comparing the concentrations of the all estimated hormones of the treated plants shows that the treatment with lower rate (40 min.) of laser rays considerably increased IAA, GA_3 and ABA concentrations over control treatment under normal and PEG conditions up to 8% PEG level. Such accumulations were decreased under all levels of PEG as a result of higher irradiations treatments (80, 120 and 160 min.)) and 12% as well as 16% PEG levels with lower rate of laser rays treatment . The treatment of 40 min. laser rays could exceed accumulations of IAA, GA_3 and ABA over the respective values of control treatment (Mean T); meanwhile the other irradiation

treatments exhibited an opposite trend. In this concern, Wilkinson (1994) elucidated the effects of radiation on plant metabolic sites which are included the synthesis of DNA, enzymes, amino acids, proteins and auxins, in addition to photosynthesis, but the same author indicated that auxin synthesis seems to be the most sensitive non-genetical process affected by irridation, thus the resultant altered concentration of auxim would be logical cause of many secondary effects.

Accordingly, it could be postulated that the lower dose (40 min.) of laser rays treatment seems the most suitable one for enhancing plant growth and development through stimulation of auxin biosynthesis. Moreover, Ghallab and Nesiem (1999) working on wheat, reported that positive response of IAA and GA_3 synthesis to 5 K rad treatment, indicating that the lower dose of gamma rays (5 K rad) seems to be the most suitable one for enhancing plant growth and development through stimulation of auxin biosynthesis.

For ABA, Maslenkora *et al.*, (1993) reported that ABA level increased with salinity stress, and that this level correlated with plant resistance to the salt stress. Also, Hatung (2004) considered that ABA is the primary hormone that mediates plant responses to stress such as cold, drought and salinity; thus is endogenous level increased with water stress.

E- Juice quality characters :

Comparing the results obtained in Table 8, clearly revealed that Juice quality characters of sugarcane plants at harvest stage, i.e. percentage of sucrose, purity, sugar recovery and total soluble solids significantly decreased with increasing PEG level (Mean P). Similar results were reported by Srivastava *et al.* (1997), Robertson *et al.* (1999) and Wiedenfeld (2000) on sugarcane plants under water stress conditions. In this respect, Kathiresan (2000) reported that water stress reduces Juice quality characters of sugarcane cv. CoC 671 and Co 6304. Moreover, Hussain *et al.* (2004) indicated that Juice quality characters (percentage of sucrose, purity, sugar recovery and total soluble solids) of sugarcane cv. CP-77-400 and COJ-84 decreased with increasing salinity levels.

When consider the mean value of all Juice quality characters of sugarcane plants at harvest stage due to each treatment (Mean T) regardless of the PEG level (Table 8), it could be noticed that the Mean T of the lower dose (40 min.) of laser irradiation which recorded significant increases of all Juice quality characters of sugarcane plants at harvest stage over the respective Mean T of the control treatment (0.0 min.); meanwhile other irradiation treatments (80, 120 and 160min.) exhibited an opposite trend. This clearly indicates that the highest doses of laser rays have an inhibitory effect on the Juice quality characters of sugarcane plants. Comparing the effect of the interaction between irradiation treatments and PEG levels clearly reveal that at 4% and 8% PEG levels, treatment with lower rate (40 min.) of laser rays produced significantly higher values of the studied Juice quality characters when compared with the control treatment, while at 12% and 16% PEG levels, a negative trend were elicited. Also, at the higher doses (80, 120 and 160 min.) of laser rays under all PEG levels, all Juice quality characters of sugarcane at harvest stage were not significantly changed as compared with the control

treatment. This strongly emphasized the superiority of laser rays at lower dose (40 min.) in stimulating to improve Juice quality characters of sugarcane plants as previously reported for simulative effects on growth characters (Table 2 and 3) and accumulated much more concentrations of the protective compound (Table 4), photosynthetic pigments (Table 5), minerals (Table 6) as well as endogenous phytohormones (Table 7) of sugarcane grown *in vitro* and *ex vitro* under different levels of PEG up to 8% PEG level.

Increasing quality characters induced by laser rays under water stress conditions was previously recorded by cholakov and Uzurovw (1997) with cucumber.

Table 8: Effect of laser irradiation doses on Juice quality characters of sugarcane plants (at harvest stage) grown in glasshouse conditions under the same different levels of PEG (Combined analysis for two seasons).

Laser irradiation doses (minutes)	Juice quality characters											
	Sucrose (%)						Purity (%)					
	PEG levels (%)					Mean (T)	PEG levels (%)					Mean (T)
	0	4	8	12	16		0	4	8	12	16	
0	18.19	17.59	16.37	15.15	14.38	16.34	86.41	80.53	75.78	71.85	67.54	76.42
40	21.25	19.76	18.89	13.91	13.08	17.38	91.69	87.19	83.23	68.75	64.19	79.01
80	17.09	16.39	14.97	12.96	12.43	14.77	81.23	76.23	71.38	63.94	60.93	70.74
120	16.51	15.09	14.19	12.18	11.68	13.93	75.61	72.59	68.55	60.11	57.28	66.83
160	15.48	14.85	13.65	11.71	10.21	13.18	71.19	68.78	64.16	57.68	53.41	63.04
Mean (P)	17.70	16.74	15.61	13.18	12.36		81.23	75.26	72.62	64.47	60.67	
L. S. D. at 5%	T = 0.29 P= 0.52 T*P= 0.98						T = 0.98 P= 1.56 T*P= 2.96					
	Sugar recovery (%)						Total soluble solids (%)					
0	11.25	10.46	9.99	9.64	8.87	10.04	18.55	17.07	15.19	14.28	12.72	15.56
40	13.55	12.33	11.65	8.53	8.01	10.81	20.31	19.52	18.38	13.11	11.61	16.59
80	10.11	9.31	8.88	8.02	7.89	8.84	17.48	16.93	14.11	11.94	10.68	14.23
120	9.41	8.92	8.53	7.69	7.25	8.36	16.31	15.71	12.56	10.77	9.55	12.98
160	9.03	8.38	7.76	7.21	6.96	7.87	14.92	12.67	11.05	9.89	8.22	11.35
Mean (P)	10.67	9.88	9.36	8.22	7.79		17.51	16.38	14.26	11.99	10.56	
L. S. D. at 5%	T = 0.27 P= 0.45 T*P= 0.85						T = 0.14 P= 0.25 T*P= 0.47					

3- Yield Components:

The results in Table 9 represent the different values of yield components; cane and sugar yield / plant (g) of regenerated sugarcane plants (8-monthes old) grown in glasshouse conditions under the same different levels of PEG. The obtained data clearly show the dramatically reduced yield components by increasing PEG levels (Mean P).

These results are in agreement with those obtained by Srivatava *et al.* (1997), Robertson *et al.* (1999) and Wiedenfeld (2000) on sugarcane, Cholakov and Uzunow (1997) on cucumber and Rashad and Ragab (2003) on grain sorghum. The reductions in the number of leaves per plant (Table 3) and photosynthetic pigments concentration (Table 5) due to PEG should have affected the photosynthetic capacity of the plant and thus reflect upon the great reduction obtained in yield components. Moreover, declines in the activities of endogenous auxins and gibberellins would account much for reduction in the yield components (Table 7). In this respect, many workers suggested that

the reduction in plant yield due to water stress may be attributed to restricts the absorption of water by plant roots and water use efficiency (Rengel, 1999) and / or imbalance in phytohormone levels through its effect on either the biosynthesis or the destruction of the plant hormones (Pessaraki, 1994).

Table 9: Effect of laser irradiation doses on yield components of sugarcane plants (8 months old) grown in glasshouse conditions under the same different levels of PEG (Combined analysis for two seasons).

Laser irradiation doses (minutes)	Cane yield / plant (g) PEG levels (%)					Mean (T)	Sugar yield / plant (g) PEG levels (%)					Mean (T)
	0	4	8	12	16		0	4	8	12	16	
Control	855.32	796.28	701.55	599.78	492.86	689.16	96.22	83.29	70.08	57.82	43.72	70.23
40	910.54	848.83	775.18	665.61	458.46	731.72	123.38	104.66	90.31	56.78	36.72	82.37
80	769.21	705.36	651.39	579.42	403.14	621.70	77.77	65.67	57.84	46.47	31.81	55.91
120	663.35	623.27	572.88	492.51	342.58	538.92	62.42	55.59	48.87	37.87	24.84	45.92
160	557.16	519.69	489.99	421.57	301.89	458.06	50.31	43.55	38.02	30.39	21.01	36.66
Mean (P)	751.12	698.69	638.19	551.78	399.79		82.02	70.55	61.02	45.87	31.62	
L. S. D. at 5%	T = 8.66 P= 14.16 T*P= 26.59						T = 1.05 P= 1.72 T*P= 3.23					

Also, the results in (Table 9) show the significant effect of low dose (40 min.) of laser rays in increasing yield components of regenerated sugarcane plants over control under normal or water stress conditions up to 8% PEG level; meanwhile completely opposite trends were obtained with other irradiation (80, 120 and 160) treatments under all PEG levels and 12% as well as 16% PEG levels for 40 min. treatment. Hence, the clear superiority of low dose of laser rays in inducing the highest degree of PEG tolerance and consequently water stress tolerance of the regenerated sugarcane plants could be clearly seen and which even surpassed over untreated control (Mean T); meanwhile the other irradiation treatments exhibited an opposite trend. As for the highly promoting effects of the low dose (40 min.) of laser rays on productivity of regenerated sugarcane plants and endogenous growth substances (Table 7), this treatment showed the greatest growth (Tables 2 and 3), protective substances (Table 4), photosynthetic pigments (Table 5), Juice quality characters (Table 6) and yield (Table 9). This result needs further investigation in a broad scale of pot experiments and in the field.

In this connection, Zubal (1990) stated that laser rays have been used to produce beneficial effects on the yield of cereals and legumes. Moreover, WoJcik (1994) working on the effects of seed irradiation with laser on the yield and chemical composition of sugar-beet roots found that treatment had a beneficial effect on yields and root yield was greatest after 1 period of exposure (54.6 tons / ha.). Furthermore, Ghallab and Omer (1998) reported that the grains of wheat presowing irradiation with ultraviolt laser rays (100 shot) significantly increased grain yield.

CONCLUSION

A wide survey of all foregoing results in the present study clearly revealed that the results obtained during the two seasons confirmed the absolute superiority of the low dose of laser rays (40 min.) compared either with untreated control treatment or with the other irradiation treatments (80, 120 and 160 min.). These results emphasized its superiority in inducing the higher degree of PEG tolerance and consequently growth of sugarcane grown *in vitro* and *ex vitro* tolerant up to 8% PEG level and which were able to continue their growth till maturing and even attained cane production. Such high degree of tolerance exhibited by low dose of laser rays (40 min.) treatment was positively associated with higher accumulation of endogenous hormonal status (IAA, GA_3 and ABA), protective substances (sugars, proline, amino acids and total soluble phenols) and photosynthetic pigments, i.e. chlorophylls (a, b and total) and carotenoids in the sugarcane grown *in vitro* and *ex vitro*. These accumulations were positively correlated with the increase in PEG levels in the medium. This in addition to the considerable increases in the Juice quality characters in the treated sugarcane plants at harvest stage. This is also applied to the considerable accumulations of much more quantities of inorganic osmotica, i.e. N, P, K, Ca and Mg in the sugarcane grown *in vitro* and *ex vitro*.

The obtained data of low dose of laser rays (40 min.) treatment during the two seasons offered strong evidence for the absolute superiority of such treatment in inducing higher degree of tolerance to PEG through the accumulations more quantities of the protective solutes compared even with the untreated control. Moreover, such behavior in the treated plants of the low dose of laser rays treatment evidently increased their ability to countract PEG stress, thus were able to keep better performance against PEG till harvest. This was reflected on a significant increment in the can yield / plant over the respective yield of all other treatments up to 8% PEG level.

The obtained data suggested that the lower dose of laser rays (40 min.) may be successfully applied to improve water stress tolerance of economic crops such as sugarcane, but it must be applied widely and after precise study with each crop to approach its optimal effectiveness in improving tolerance to water stress.

Further genetical as well as physiological studies are needed at the cell level to disclose whether, the role of laser rays in regulating the uptake and accumulations of different solutes, is attributed to some alteration in the properties of the cell membranes or to any other genetic changes or somatic mutation.

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بعض الدراسات الفسيولوجية على زيادة تحمل نباتات القصب المكاثرة معمليا للاجهاد المائى باستخدام أشعة الليزر

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أجريت تجارب خلال موسمين متتاليين ٢٠٠١، ٢٠٠٢ لدراسة تأثير أشعة الليزر (الأرجون) بجرعات مختلفة (صفر ، ٤٠، ٨٠، ١٢٠، ١٦٠ دقيقة) فى تحسين مقاومة الجفاف لنباتات القصب المكاثرة معلميا وذلك تحت تأثير مستويات مختلفة من البولى ايثيلين جليكول (صفر ، ٤ ، ٨ ، ١٢ ، ١٦%).

وقد ثبت من نتائج البحث أن معاملة ٤٠ دقيقة أشعة الليزر أدت للحصول على زيادة معنوية فى صفات النمو لنباتات القصب الصغيرة النامية فى المعمل (النسبة المئوية للمتبقى ، طول الافرع ، عدد الاوراق والجذور ، الوزن الطازج والجاف للمجموع الخضرى) وصفات النمو لنباتات القصب المكاثرة معمليا والنامية فى الصوبة (طول الساق ، سمك الساق ، عدد الاوراق والوزن الجاف للمجموع الخضرى) والمحصول (محصول القصب والسكر لكل نبات) وصفات الجودة للعصير (النسبة المئوية للسكروز و النقاوة وناتج السكر والمواد الصلبة الذائبة الكلية) مقارنة بالكنترول الغير معامل وحتى ٨% بولى ايثيلين جليكول.وأيضا المعاملة بالجرعة المخفضة (٤٠ دقيقة) من أشعة الليزر قد مكنت نباتات القصب الصغيرة والناتجة من القمة الخضرية المعاملة من مقاومة الاجهاد المائى حتى مستوى ٨% بولى ايثيلين جليكول فظلت قادرة على الاستمرار فى النمو فى الصوبة حتى مرحلة النضج وإنتاج محصول القصب.

أظهرت أيضا نتائج التحليل الكيماوى لأنسجة نباتات القصب الصغيرة النامية فى المعمل والمجموع الخضرى لنباتات القصب المكاثرة معمليا والنامية فى الصوبة أن تحمل الاجهاد المائى والذى ظهر بدرجات عالية فى المعاملة ٤٠ دقيقة أشعة الليزر قد ارتبط ارتباطا موجبا بتراكم كميات كبيرة ومتزايدة من عناصر النيتروجين والفوسفور والبوتاسيوم والمغنيسيوم والكالسيوم وتركيز منخفض من الصوديوم، وذلك بالإضافة إلى تراكم كميات كبيرة من السكروز والبرولين والاحماض الامينية والفينولات الذائبة الكلية وصبغات البناء الضوئى (كلوروفيل أ ، ب والكاروتينويدات) و الهرمونات النباتية (الجبريللينات والاوكسينات وحمض الابسيسيك) بمستويات تزايدت طرديا بزيادة مستوى البولى ايثيلين جليكول فى البيئة.

يتضح من النتائج المتحصل عليها من هذا البحث إمكانية استخدام أشعة الليزر (الارجون) بنجاح فى تحسين صفة تحمل الجفاف فى المحاصيل الاقتصادية مثل قصب السكر.