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ORIGINAL ARTICLES

Effect of Different Laser Wavelength on Callus of Date Palm

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ABSTRACT

The present work was carried out to investigate the effects of red diode laser at wavelength 650 nm power 7 mw and green diode laser at wavelength 532 nm power 160 mw on palm callus culture. The callus were exposed to red laser for 5, 10, 15 and 20 minutes and 4,8,12 and 16 minutes to green laser. In most cases the highest content of total soluble amino acids and total phenols were obtained from the treatment of 20 min in red laser and treatment of 16 min in green laser. The highest values of Total soluble sugars, Total Indol were obtained from the treatment of 10 min in red laser and treatment of 8 min in green laser. Electrophoreses protein and DNA analysis was carried out to study the genetic similarity between different time of Laser irradiation with palm callus cultures. DNA analysis which performed a great similarity between control and 10 min treatment reached to 92% while Lower similarity between control and 15 min treatment reached to 84%

Key word: Laser, red Laser, green Laser, diode Laser, palm, Callus cultures.

Introduction

Date palm (*Phoenix dactylifera L.*) is one of the most economically important dioecious perennial plants in arid areas of the Middle East and North Africa; Awad (2007). Moreover, it successfully tolerates extremely adverse environmental conditions, including drought, high temperature and salinity, which are the peculiar criteria of desert lands. It makes a significant contribution toward the creation of equable microclimates within the fragil oasis ecosystems, thus enabling sustainable agricultural development in many drought and saline affected regions Barreveld (1993). Plant tissue culture technique is known as a promising method for the mass propagation of date palm .

Light, which is closely related to a gain of energy and metabolic processes, is usually an important factor affecting growth, organogenesis, and the formation of plant products including both primary and secondary metabolites. There are number of reports concerning elevation of the activity of gene products and the enhancement of secondary metabolites by light irradiation. Kurata *et al*. (1998) reported that intermittent light irradiation enhances caffeine biosynthesis by Coffea arabica cells, and Makunga *et al*. (1997) found that light irradiation enhances anthocyanin production in Oxalis reclinata calli. In the hairy root cultures of Artemisia. annua L., it was found that the biomass of hairy roots and artemisinin content under red light were 17 and 67% higher than those obtained under white light Wang *et al*. (2001). Light quality had a marked effect on differentiation and physiological and biochemical process of cultured cucumber and tomato calli. Activities of peroxidase (POD) and phenylalanine ammonia-lyase(PAL) were examined during the culture of cucumber and tomato under white, blue, yellow, red and green lights as well in the dark. The results indicated that the changes of activities of these enzymes were positively correlated to the differentiation of tissue and organ in culture process. Red and green lights inhibited bud formation, and no third peak had been obtained. So peroxidase and phenylalanine ammonia-lyase can be considered to be marker enzymes of tissue and organ differentiation in cucumber and tomato WANG *et al*. (1991). Effects of He-Ne laser and Magnetic field on callus growth and shikonin derivatives formation in *Onosma paniculatum* Bur. Results showed that callus growth was promoted when exposed to He-Ne laser with power 0.37mw/cm2at 1h, while content of shikonin derivatives increased with 15.7% and 27.8% at 2 and 3hs. The magnetic field stimulated both of callus growth and biosynthesis of shikonin derivatives by 10KGS, 15' treatment. The contemporay and subsequent passage content of shikonin derivatives increased by 9.5% and 27.5%. The stable increase of shikonin derivatives content had been observed by Wang Mansi *et al*. (1994) .On the other hand Chen Rumin *el al.* (1992) found that laser processing can significantly be contributed to its growth and differentiation of callus, 20 MW of He-Ne laser treatment can increase the callus fresh weight than the control 170%, and 0.1W rejection of argon ion laser treatment can significantly promote bud differentiation. Study results showed that, due to the above reasons and the results of

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light and no clear relationship between quality caused by certain specific proteins produced by laser-related . while Zhang Minghe *et al*. (1993) show that callus of rice using laser treatment can make the Green plant differentiation rate significantly higher, giving very good differntiation in seedling growth .Zhang Kezhong *et al*. (1994) using method domestic DATACHROM-5000 type dye Pulse Laser processing grape "victory" varieties of more injury organization, with polypropylene n amine coagulation rubber electrophoresis method (PAGE) separation peroxide enzyme staff enzyme, The application of laser irradiation on grape growth development and peroxide enzyme staff ; showed that appropriate dose of laser irradiation can make grape more injury organization clear ahead of differentiation; laser to affect grape peroxide enzyme staff enzyme gene of regulation.

There for, the objective of this study was to investigate the effects of laser wavelength 650, 532 nm and power 7, 160 mw on palm callus culture.

Materials And Methods

This work was carried out in the Central Laboratory for Research and Development of Date Palm, Agricultural Research Center, Ministry of Agriculture and National Institute of Laser Enhanced Science Cairo University, aiming to study the effect of two different Laser irradiation types on callus of date palm C.V. Khalass obtained from explants excised from shoot tips of 6 years old seedlings. Red diode Laser at wavelength 650 nm and power 7 mw or green diode Laser at wavelength 532 nm and power 160 mw at same temperature were used at 30 cm distance. The tested irradiation durations were 5,10,15 and 20 min for red Laser, however they were 4,8,12 and 16 min for green Laser treatment can increase the callus fresh weight than the control 140%

The callus was initiated on MS-Medium (Murashige and Skoog, (1962)) .

After incubation of callus cells at 25 C in darkness for 2 month, this method can be used in plant breeding programs.

The following data had been recorded : Sugar content according to Thomas and Ducher (1924) .Free amino acids according to Moore and Stein (1954). Phenolic compounds by using the colormetric method described by Snell and Snell (1953). Total indoles according to Larsen *et al*. (1962).

2.1 Molecular studies:

1-Protein electrophoresis analysis (SDS-PAGE) – Polyacrylamide Gel Elecctrophoresis according to Laemmli (1970):

2– DNA Fingerprints DNA Fingerprints according to Porebski *et al*. (1977).

2.2 Statistical analyses:

All statistical analyses were carried out SAS 10 version software. R^2 and pr>f descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples.

p-Values <0.05 were considered significant (Table 1,2).

Result:

The data shown in Tables 1&2 revealed that application of Laser on callus of date palm affected significantly on increasing the contents to total soluble amino acids in callus treated for 20 min for the application of Laser diode 650 nm, resulting in (0.223), which is more than double value for the untreated callus (0.108). However it increased with prolonging the duration of irradiation .The application of Laser diode 532 nm irradiation affected cleary on increasing the contents from 0.108 for control to reach its maximum value 0.988 for 16 min ; the increases occurred gradually, showing sharp rate after 16 min. treatment (Table 2).This result agreed with that reported by Satter *et al*. (1990), Who obtained increases in essential and non essential amino acids of soybean heated with 0.10kgy. However many factors affect the role of ionizing irradiation on amino acids such as sensitivity of the exposed system , type of particular functional tissue, aqueous soaking after irradiations siddhuraju *et al*. (2002).

Concerning total soluble sugars the data in (Table 1) showed that ; at the different treatment periods the irradiation with 650 nm Laser diode an increased values had been obtained giving its maximum content 1.37 after 10 min irradiation, then decreased slightly with prolonging the duration of application, compared with the untreated callus 0.396 .

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Table 1: Effect of Laser diode 650 nm irradiation treatments on the contents of total soluble amino acids, total soluble sugars, total phenols and total Indols in palm callus cultures.

Treatments (min)	Total soluble amino acids	Total soluble sugars	Total phenols	Total Indol
0	0.108	0.396	0.104	0.169
5	0.083	0.646	0.063	0.34
10	0.186	1.37	0.266	0.56
15	0.200	1.36	0.405	0.548
20	0.223	1.335	0.582	0.55
Anova	0.809	0.775	0.91	0.775
R^2	0.037	0.048	0.011	0.048
Pr>F				

Table 2: Effect of Laser diode 532 nm irradiation treatments on the contents of total soluble amino acids, total soluble sugars, total phenols and total Indols in palm callus cultures .

Treatments (min)	Total soluble amino acids	Total soluble sugars	Total phenols	Total Indol
0	0.108	0.396	0.104	0.169
4	0.131	0.456	0.061	0.271
8	0.355	0.593	0.144	0.462
12	0.409	0.578	0.196	0.445
16	0.988	0.585	0.267	0.450
Anova	0.821	0.772	0.824	0.772
R^2	0.033	0.049	0.033	0.049
Pr>F				

With the application of Laser diode 532 nm the highest content (0.593) was recorded for that treated for 8 min, followed by (0.578) at 12 min, and (0.585) at 16 min, while control treatment resulted in 0.396 (Table 2). The effect depends on its role in controlling cellular metabolism in addition to its storage function, these results cleared that total amino acids and soluble sugared are significant contributors to metabolism under Laser irradiation condition.

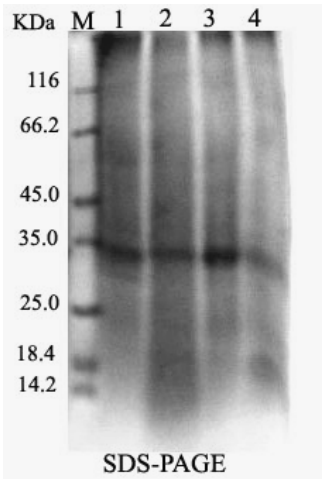


Fig (1)

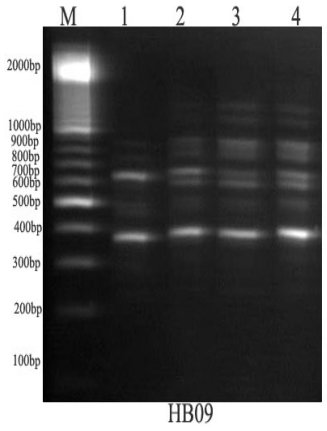
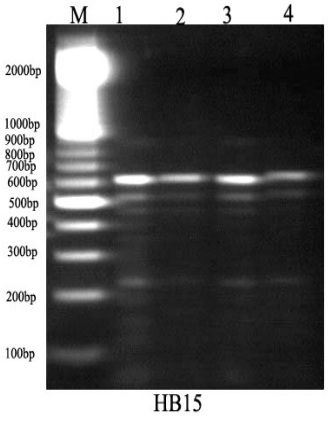


Fig (2)



Fig(3)

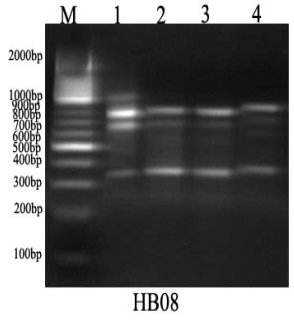


Fig (4)

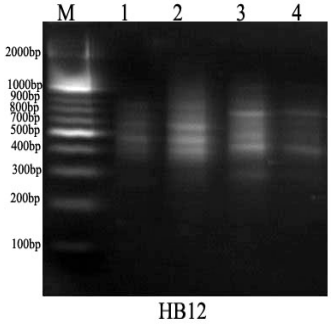


Fig (5)

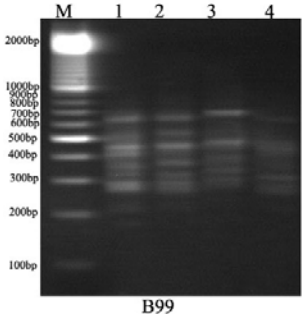


Fig (6)

The variation in total phenols shown in (Table 1). The data indicates a significant ($p<0.05$) increase in total phenols content in irradiated palm callus cultures as compared to control. After irradiation, total phenols content for control treatment was (0.104) and reached its highest value (0.582) at 20 min duration (Table 1). Also content for unirradiated sample was (0.104) and reached its maximum value (0.267) at the longest period of irradiation treatments 16 min (Table 2).

2.Molecular studies in palm callus cultures

A. Protein analysis:

Electrophoreses protein analysis had been carried out to study the genetic similarity at different duration of Laser irradiation for palm callus cultures. It showed that 42.8 % Monomorphic and 7 total band had bean obtained (Figure 1). Molecular weight 29 KDa was not found in the callus treated for 5,10 and 15 min, While Molecular weight 13 KDa had not been obtained at in 10 and 15 min.

Molecular weight 20 KDa had not been detected at 10 min (Figure 1).

B. DNA analysis:

DNA analysis ; by using five primers for the similarities between the three Laser durations of irradiation for palm callus cultures as shown in (Figure 6), revealing that B99 primer display Molecular weight (450bp) in fingerprint for 5 min and 10 min had not been detected ; while Molecular weight (180bp) was not found in that treated with 5,10 and 15min and Molecular weight (225bp) had been displayed only at 5 min. In (Figure 2) primer HB09 Molecular weight of (530, 920 and 1050bp) were absent for control but visible at 5,10 and 15 min. (Figure 3) showed that primer HB15 for Molecular weight of (140 and 230bp) were not detected at 5,10 and 15 min, while Molecular weight of (400 and 450bp) were not found at 5min, Molecular weight of (945bp) was not detected at 5 and 15 min. (Figure 4) showed that primer HB08 displayed Molecular weight of (1020bp) only in control. Primer HB12 at (Figure 5) displayed Molecular weight of (790 bp) in fingerprint of palm callus cultures was found only in control and 5 min ;while Molecular weight 760bp was not detected in 15 min.

DNA analysis had been also carried out to find the similarities between palm callus and Laser irridation. The results in (Table 3) illustrated DNA analysis, which performed a great similarity between control and 10 min treatment reached to 92%. Lower similarity between control and 15 min treatment reached to 84%. While similarity between control and 5 min treatment reached to 90%.

Table 3: DNA analysis similarity between different Laser duration

Treatment	Control	5 min	10 min	15 min
Control	------	90%	92%	84%

Discussion:

The Increase in total phenols and total flavonoids contents in irradiated plants had been reported by Lee *et al*. (2009). Such increase in total phenols and total flavonoids is due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by gamma irradiation as suggested by Harrison and Were (2007). Irradiation exerts its effects as direct and indirect mechanisms. In case of indirect mechanism, radiolysis of water results in the production of free radicals such as hydroxyl radicals, hydroperoxide radicals and hydrated electrons.These radicals may break the glycosidic bonds of procyanidin trimer, tetramer and hexamer that are present in plants, leading to the formation of procyanidin mono- mers, which increase the total phenolic and total flavonoids content in irradiated plants Lee *et al*. (2009). In plant tissues many phenolic compounds are potential antioxidants: flavonoids, tannins and lignin precursors may act as ROS (reactive oxygen species) scavenging compounds. Observed increase in total phenolic and tannin contents was beneficial for antioxidant proper ties of irradiated soybean seeds due to polymerization of phenolic constituents and also cross-linking and fragmentation, which were the key reactions controlling the properties of macromolecules such as proteins (Tagawa *et al*. 2004, Stajner *et al*. 2007). These results are in harmony with that reported by Mahmoud (2002), Nassar *et al*. (2004) and Moussa (2011) who reported an increase in carbohydrates and soluble sugars in response to plant irradiation. The increase in total soluble sugars might be attributed to degradation of starch fractions Akulova *et al*. (1970). Data in (Table 1). indicated that sugar accumulation has some role in the osmotic adjustment production. It has been suggested that the function of accumulated soluble sugars under water stress can occure in two ways, which are difficult to separate: as osmotic agents and as osmoprotectors Bohnert *et al*. (1995). As osmoprotectors, sugars stabilize proteins and membranes, most likely substituting the water in the formation of hydrogen bonds with polypeptide polar residues; Crowe *et al*. (1992), and phospholipid phosphate groups, Strauss and Hauser (1986). There is no doubt

that total soluble sugars exert a positive role in the alleviation of the imposed stress via osmotic adjustment in plants (Kameli and Losel, 1996 , Kerepesi and Galiba, 2000). Some of the soluble sugars accumulate in stressed cells to maintain membrane phospholipids in the liquid crystalline phase and to prevent structural changes in soluble proteins and others play a key role in stress induced metabolic processes Kerepesi and Galiba (2000). Substantial osmotic adjustment was observed in adapted soybean cell suspension cultures exposed to water stress, mainly due to increased glucose, fructose and sucrose ; Dubey and Pessarakli (1995). Although the content of total soluble sugars nonsignificantly changed by the application of gamma-irradiation alone, it increased in much pronounced levels in response to a combined stress of irradiation and drought than in response to drought stress alone. This behavior confirms that increasing osmolyte/osmoprotectant contents may come as another defensive mechanism comes via the activation of genes responsible for the expression of the enzymes involved in the accumulation of these osmolytes, which caused by the pre-exposing to gamma rays, leading to more protection for the upregulating enzymes involved in the anabolism of these contents

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