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ORIGINAL PAPER

Effect of red and blue light emitting diodes "CRB-LED" on in vitro organogenesis of date palm (*Phoenix dactylifera* L.) cv. Alshakr

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Abstract The objective of the present study is to determine the effect of light source on enhancement of shoot multiplication, phytochemicals, as well as, antioxidant enzyme activities of in vitro cultures of date palm cv. Alshakr. In vitro-grown buds were cultured on Murashige and Skoog (MS) medium and incubated under a conventional white fluorescent light (control), and combinations of red + blue light emitting diode (18:2) (CRB-LED). Results revealed that the treatment of CRB-LED showed a significant increase in the number of shoots compared with the white florescent light. Total soluble carbohydrate "TSCH" (7.10 mg g^{-1} DW.), starch (1.63 mg g^{-1} DW.) and free amino acids (2.90 mg g^{-1} DW.) were significantly higher in CRB-LED ($p < 0.05$). Additionally, CRB-LED induced a higher peroxidase activity (25.50 U ml^{-1}) compared with the white fluorescent light treatment (19.74 U ml^{-1}) as control treatment. Potassium, magnesium and sodium contents in (3.62, 13.99 and 2.76 mg g^{-1} DW.) were increased in in vitro shoots under CRB-LED treatment in comparison with fluorescent light ($p < 0.05$). Protein profile showed the appearance of newly bands with the molecular weight of 38 and 60 kDa at the treatment CRB-LED compared with control treatment. Our results demonstrate the positive effects of CRB-LED light during the course of date palm tissue cultures.

Keywords Light-emitting diodes (LEDs) · Multiple shoots · Macronutrients · Antioxidant enzyme · Protein patterns

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Introduction

Date palm (*Phoenix dactylifera* L.) of the family Arecaceae is a key plantation crop in many countries around the world, including of arid regions of West Asia and North Africa (Al-Khalifah et al. 2013). Traditional method of date palm propagation is through offshoots that usually grows underground or near the surface beside the trunk of tree (Al Khateeb and Ali-Dinar 2002). The limited availability of offshoots renders this traditional propagation method inadequate, particularly some cultivars do not produce offshoot at all or have very few cultivable offshoots (10–30) (Heselmans 1997; Reda et al. 2011). Tissue culture multiplication technique could be considered as one of the most efficient tool for the development of the date palm culture. The benefits of using this technique are production of many plantlets from one date palm mother tree, pathogen-free and true-to-type (Al Khateeb and Ali-Dinar 2002; Bekheet 2013).

For plantlets production, the incubation and propagation of the explants are only performed under light conditions, with propagation being the main step for research and commercial production (AlKhateeb 2008). Several external and internal factors regulate in vitro plant growth and development among them, the light is most important. The light source generally used for tissue culture is broad-spectrum fluorescent lamps, which have peak wavelengths from 380 to 750 nm (Kim et al. 2004). Recently, Light emitting diodes (LEDs) as a new light source have drawn a considerable attention as an alternative light option for in vitro propagation, and indicated that LED have many advantages over conventional fluorescent lights, including specific wavelength, low thermal output, adjustable light intensity and quality, high photoelectric conversion efficiency as well as small size and extended longevity (Morrow 2008; Rocha et al. 2010).

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The effects of LED light sources on several plants as maize, cotton and peas have been reported, and its suitability have been proven for these plants (Felker et al. 1995; Huimin et al. 2010; Wu et al. 2007). LEDs have been used for inducing organogenesis, and promoting or enhancing the growth of plantlets in vitro and ex vitro in several horticultural commodities. Many recent researches proved the nutrient and growth regulators (type and concentration) effect on the in vitro propagation of date palm. However, research on the effect of light source on date palm micropropagation is rather limited.

Therefore, the purpose of this study was to evaluate the effects of the CRB-LED and white fluorescent light on direct organogenesis by induction of adventitious buds from shoot tip and multiplication shoots of date palm cv. Alshakr in vitro.

Materials and methods

Plant material and sterilization

Young offshoots (2–3 years old) of date palm cv. Alshakr were detached from mother palm. Outer leaves and fibrous tissues at their bases were removed gradually until exposure of the shoot tip zone. Sheathing leaf base enclosing the very young leaves of the heart of the offshoot was left in place to protect it from disinfection solutions. The explants were taken and kept in antioxidant solution (100 mg l^{-1} ascorbic acid + 150 mg l^{-1} citric acid). Sterilization of explants was performed using 70 % ethanol for 1 min and 2.5 % sodium hypochlorite for 20 min. Explants were then rinsed three times with sterile distilled water. The shoot tip terminal, about 1 cm long, was sectioned longitudinally into four sections. In order to induce bud formation, explants were transferred to Murashige and Skoog (1962) (MS) basal medium supplemented with 2 mg l^{-1} 2ip, 1 mg l^{-1} NAA, 3 g l^{-1} activated charcoal, 100 mg l^{-1} glutamine, 5 mg l^{-1} thiamine HCl, 1 mg l^{-1} biotin, 30 g l^{-1} sucrose, and solidified with agar at 6.0 g l^{-1}.

All the media were adjusted to pH 5.8 with 0.1 N NaOH or HCl, before the addition of agar. Media were dispensed into culture test tubes. Subsequently autoclaved at 121 °C and 1.04 kg cm^{-2} for 15 min. Cultures were kept under complete darkness at 27 ± 2 °C. The cultures were transferred to fresh media, with the same composition after every 5 weeks interval until the buds had initiated. For multiplication and growing, the budding tissues formed were cut into two to three segments and subcultured on multiplication medium supplemented as mentioned above, with the exception of plant growth regulators 1 mg l^{-1} (NAA), 0.5 mg l^{-1} (BA) and 0.5 mg l^{-1} kinetin (K). Cultures were then incubated at 27 ± 1 °C with 14/8-h photoperiod (light/dark) provided by light treatment consisting of CRB-LED with ratio 18 red:2 blue at the light intensity of (~80–100 µmol m^{-2} s^{-1}) and white fluorescent lights as a control of same intensity ~20–25 µmol m^{-2} s^{-1} (Fig. 1a-T1 and AT2, b). Subcultures were made at 5 week intervals on the same medium to obtain shoots. The response of developed buds to shoot (%), and shoot number per jar were calculated.

Determination of biochemical parameters in treated shoot

Dry weight samples of the shoots (0.05 g) were placed into 15 ml tubes and then 5 ml of distilled water was added and mixed in. The supernatant was collected after 30 min in a water bath at 85 °C. This step was repeated once and then distilled water was added to obtain 10 ml of the extract for use in determining total carbohydrates and free amino acid contents (mg g^{-1} DW). The total carbohydrates content was determined using the sulfuric acid anthrone method at a wavelength of 630 nm Morris (1948). Amino acid

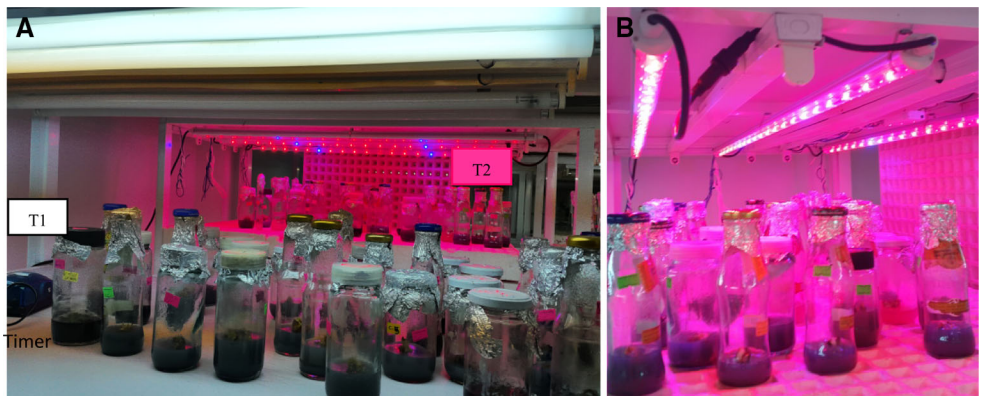

Fig. 1 Light-emitting diodes (LEDs) and white fluorescent lamps mounted on the ceiling of growth chambers on multiplication of adventitious shoots of date palm cv. Alshakr in vitro. **a** *T1* white fluorescent lamps, **a** *T2* and **b** CRB-LED

content was determined using the ninhydrin method at a wavelength of 570 nm Moore and Stein (1948). Starch was extracted according to the procedures from Takahashi et al. (1995).

Macronutrients content in in vitro grown shoots

Content of total nitrogen, phosphorus, potassium, calcium, magnesium and sodium in shoots was analyzed according to the method described by Cresser and Parsons (1979). 0.2 g of shoot samples (as dry weight) were taken into a caldal flask with a capacity of 100 cm^3 and digested with a mixture of sulfuric acid (69 %) and perchloric acid (62 %) under heating for one hour, subsequently, the digested solution was transferred into volumetric flask 50 cm^3, and volumes were completed in size with distilled water. Chemical analyses were performed using the following methods: total N was measured—by the distillation method according to Kjeldahl (Page et al. 1982), Phosphorus was measured by spectrophotometer at 880 nm, according to Murphy and Riley (1962). K, Ca, Mg, and Na were determined by atomic absorption spectrometry, according to method described by Black (1968).

Estimation of peroxidase activity

Enzyme extract from the shoots was prepared as recommended by (Maxwell and Bateman 1967). The leaf tissues were grounded with 0.1 M sodium phosphate buffer at pH 7.1 (2 ml buffer/g of fresh shoot tissues), in a mortar. These triturated tissues were strained through four layers of cheesecloth and the filtrates were centrifuged at 3000 rpm for 20 min at 6 °C. The supernatant fluid was used for enzyme assays. Peroxidase activity was estimated according to the method of Allam and Hollis (1972).

Extraction of protein and gel electrophoresis

Extraction of protein and gel electrophoresis

Proteins were extracted by homogenizing the 0.333 gm freeze-dried shoot sample in pre-chilled mortar and pestle using 1 ml of extraction buffer consisting of 0.2 M, tris hydroxymethyl aminomethane (Tris); 0.001 M ethylene diamine tetra acetic acid (Na2 + EDTA); 12 %, glycerol; 0.01 M, dithiothreitol (DTT); and 0.05 mM phenyl methyl sulfonyl fluoride (PMSF). The samples were centrifuged at 15,000×*g* for 15 min, and the supernatant was used for determination of total protein content. The protein sample was added with an equal volume of cracking buffer (0.125 M Tris HCl pH 6.8; 4 % SDS; 20 % glycerol; 10 % β-mercaptoethanol and 0.01 % bromophenol blue) and was denatured by boiling in waterbath at 90 °C for 3 min. Protein samples (~500 µg) were electrophoresed in a discontinuous SDS polyacrylamide gel following Laemmli (1970) using a 12 % resolving gel (0.375 M, Tris. Cl; pH 8.8) and 4 % stacking gel (0.125 M, Tris–Cl; pH 6.8) in Tris–glycine buffer (0.025 M, Tris; pH 8.3; 0.192 M, glycine; 0.1 %, SDS) for 16 h, constantly at 20 mA. Staining of the gel was done using 0.2 % (w/v) Commassie Brilliant Blue R-250 in 12.5 % (w/v) trichloroacetic acid (TCA). The position of the protein band in the gel was expressed to compare with standard protein markers with known molecular weight.

Statistical analysis

The data were statistically analyzed by one-way analysis of variance (ANOVA). The least significant difference (LSD) method was used to test the difference between treatments and $p \leq 0.05$ was considered statistically significant. Statistical analyses were performed with SPSS packet software. All treatment were replicated fifteen times.

Results

The growth and development of buds were affected significantly by different light source treatments in vitro. The highest number of buds was recorded at CRB-LED, while the lowest number of buds was recorded at white fluorescent lights (Fig. 2a, b) (data not shown).

The cultured buds showed significant variations ($p < 0.05$) in their response percentage as well as the formation of shoots undergo the influence of light source. The cultures grown under CRB-LED showed a better result in response percentage (73.34 %) of buds producing shoots and average shoots formation (8.10 ± 0.81 shoots/jar) compared with the cultures grown under the white fluorescent lights (control treatment), which were 60.0 % and 4.8 ± 0.29 shoots, respectively (Fig. 3a, b).

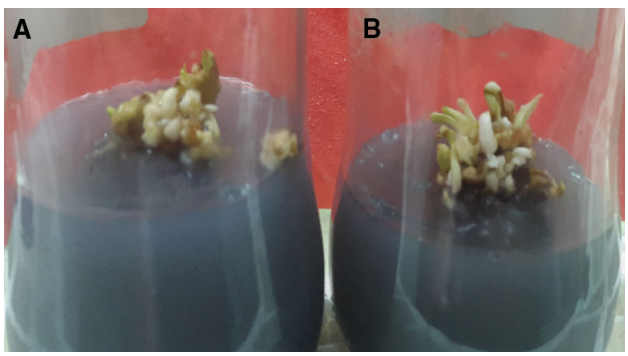

Fig. 2 Cluster of buds on multiplication medium under **a** white fluorescent lamps and **b** CRB-LED

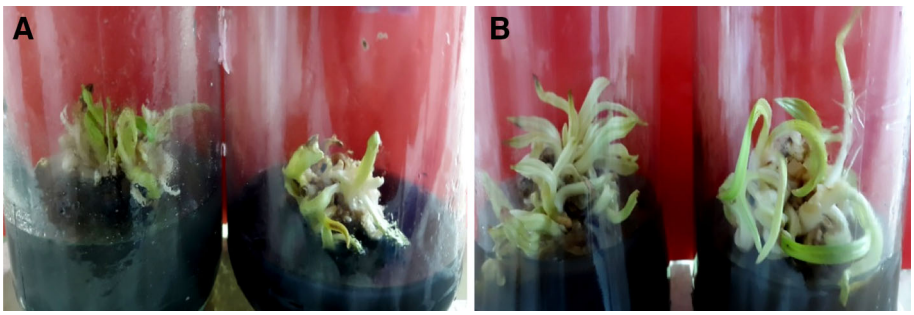

Fig. 3 Multiple shoot proliferation of date palm cv. Alshakr in vitro under **a** white fluorescent lamps, **b** CRB-LED

Table 1 The effect of light source on the carbohydrates, starch and amino acids content and activity of peroxidase in shoots of date palm cv. Alshakr in vitro

Light source	Carbohydrates (mg g^{-1} DW.)	Starch (mg g^{-1} DW.)	Amino acids (mg g^{-1} DW.)	Peroxidase activity (U ml^{-1})
White fluorescent lamps	4.83 ± 0.29 b	0.95 ± 0.08 b	1.83 ± 0.19 b	19.74 ± 1.20 b
CRB-LED	7.10 ± 0.64 a	1.63 ± 0.09 a	2.90 ± 0.30 a	25.50 ± 1.90 a

±Standard error (n15). Values followed by the same letter are not significantly different at $p < 0.05$

Table 2 The effect of light source on the macronutrients content in the shoots of date palm cv. Alshakr in vitro

Light source	N (%)	P (mg g^{-1} DW.)	K (mg g^{-1} DW.)	Ca (mg g^{-1} DW.)	Mg (mg g^{-1} DW.)	Na (mg g^{-1} DW.)
White fluorescent lamps	4.98 ± 0.38 a	4.92 ± 0.42 a	2.90 ± 0.18 b	19.04 ± 1.4 a	12.40 ± 0.50 b	2.06 ± 0.14 b
CRB-LED	5.02 ± 0.56 a	4.93 ± 0.48 a	3.62 ± 0.45 a	18.98 ± 1.20 a	13.99 ± 0.95 a	2.76 ± 0.09 a

±Standard error (n15). Values followed by the same letter are not significantly different at $p < 0.05$

Effect of light quality on some biochemical parameters

Biochemical analysis (Table 1) revealed the effect of light source on total soluble carbohydrate (TSCH), starch and amino acids in the shoots of date palm cv. Alshakr. The amount of TSCH was 47.0 % higher in shoots grown under CRB-LED compared to the fluorescent light ($p < 0.05$). Similarly, shoots grown under CRB-LED accumulated a higher amount of starch (71.6 %) compared with the fluorescent light ($p < 0.05$) (Table 1). Shoots incubated under CRB-LED showed the highest amino acid content (58.5 %) compared with the fluorescent light ($p < 0.05$); while the statistical analysis showed that the treatment of CRB-LED induced the highest peroxidase activity (29.2 %), which was significantly different than what was reported at the shoots grown under white fluorescent lights (control treatment) ($p < 0.05$) (Table 1).

The CRB-LED treatment led to some of the most significant impacts on accumulations of macronutrient in the shoot tissues of the date palm. Shoots incubated under CRB-LED caused significant ($p < 0.05$) increases in shoot tissue K (24.8 %), Mg (12.8 %), and Na (34.0 %) when compared with the fluorescent light treatment (Table 2). No significant effect of light source on shoots nutrient content was shown in regard to nitrogen, phosphorus and calcium (Table 2).

SDS-PAGE protein patterns

Protein profile was analyzed in shoots of date palm cv. (Figure 4) in order to follow any possible alterations in gene expression in shoots incubated under two light sources. It was evident that the shoots grown under CRB-LED induced the appearance of newly protein bands, specifically two new bands with molecular weights of 38 and 60 kDa appeared in comparison with the control treatment (fluorescent lights source) (Fig. 4). Whereas, the shoots grown under CRB-LED did not show any differences in their protein profile of the bands with molecular weights of 14.3, 20.1, 28.39, 43.0 and 66.9 kDa compared with shoots incubated under white fluorescent lights.

Discussion

Our results demonstrated the benefits of photosynthetically active radiation supplied with CRB-LED for the development of adventitious shoots from buds without an

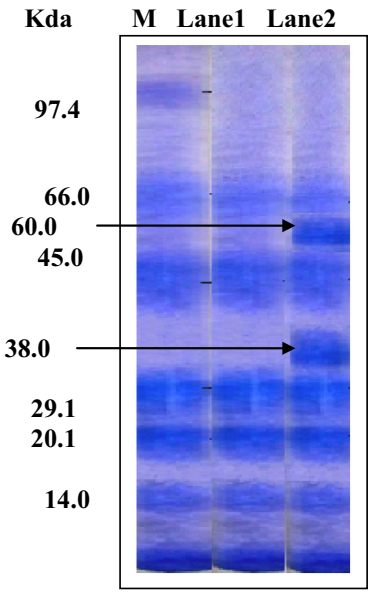

Fig. 4 SDS-PAGE of protein extracted from shoots of date palm cv. Alshakr grown under white fluorescent lamps (control) and CRB-LED." (*M* marker protein) *Lane 1* white fluorescent lamps (control), *Lane 2* CRB-LED

intervening callus phase as well as for high multiplication rates and highest shoot numbers CRB-LED treatment.

LED irradiation at some particular wavelength was found to be advantageous for micropropagation, replacing the more energy-demanding counterparts of fluorescent lights (Mengxi et al. 2011). Li et al. (2010) reported that the light-emitting diode (LED) provide an alternative to fluorescent lights especially during in vitro shoot regeneration. The banana plantlets were enhanced and compared under 80 % red:20 % blue LED (Nhut et al. 2002). It was reported that a CRB-LED enhanced plant growth compared to monochromatic LEDs (Li et al. 2010). A study by Harun et al. (2013) revealed that treatment under 16:4 red:blue ratio is more effective and increased the number of shoots and leaf.

Fluorescent lamps were used to illuminate plant tissue cultures to emit light deficient in the far-red wavelengths, thus care must be taken to provide the spectral balance suitable for growth of cultures (Wilson et al. 1993). Biswas et al. (2007) reported that the morphogenesis of strawberry tissue cultures can be manipulated by light regimes for improving efficiency of propagation. In our study, the energy distribution of CRB-LED was 90 % red and 10 % blue. It was clear from these experiments that development of date palm tissues were responsive to light and enhanced by CRB-LED light source compared with the white fluorescent lights (control). Additionally, the red light mediated responses are regulated by activation or deactivation of photoreceptors (such as phytochrome or blue light receptor), and that the transduction pathway involves irreversible switching of gene expression during early stages of development (Frederick et al. 1995). As reported by Da Silva (2014), the red:blue ratio of LEDs significantly influenced in vitro growth response of papaya plantlets, where red LED strongly stimulated the formation of shoots and a high shoot number. The positive effects of LED on shoot regeneration from the leaf explants of *Bacopa monnieri* L. Pennell was reported (Karataş and Aasim 2014).

The positive stimulation of CRB-LED light source on date palm growth was in a good agreement with several studies which revealed that the CRB-LED at the appropriate ratios enhanced the plant growth and development of *Cymbidium* (70 % red LED plus 30 % blue LED), *Musa* spp., *Eucalyptus*, *Spatiphyllium* and *Paphiopedilum* (80 % red LED plus 20 % blue LED) (Nhut 2002; Tanaka and Sakanishi 1980). The results of Abdullahil Baque et al. (2010) demonstrated that the best growth of Calanthe plantlets was obtained under the CRB-LED. Moreover, CRB-LED significantly enhanced adventitious bud formation and stem elongation in Gerbera, while blue light increased the number of adventitious buds and the fresh weight of shoots obtained from Freesia (Gabryszewska and Rudnicki 1995; Bach and Świderski 2000).

Light source also regulates carbohydrate metabolism in plants and thereby affects growth of plants (Kowallik 1987; Lefsrud et al. 2008). In the present study, we investigated the effect of spectral quality on several primary metabolites. These results revealed that CRB-LED was a suitable light source for accumulation of total soluble carbohydrate and starch in shoots of date palm cv. Alshakr than the shoots incubated under fluorescent light. Many confirmations were reported for the positive effect of CRB-LED in the accumulation of these compounds (Zheng et al. 2008). Therefore, any changes in lights not only affect total soluble carbohydrate, but they also participate in regulating important vitals. Wang et al. (2009) concluded the carbohydrates contents were consistent with the transcript levels of enzyme genes that are involved in the calvin cycle. It is well know that LED red and blue ratio can affect biochemical contents, such as total soluble carbohydrate, starch and amino acid content, thus, could be attributed to the synergistic interactions between phytochrome and blue or red light receptors (Kim et al. 2004). Light source, especially red and far red light, is known to strongly influence carbohydrate accumulation (Ranwala et al. 2002).

Carbohydrate accumulation in plants was increased in strawberries grown under CRB-LED (Giedrė et al. 2010). Red light enhanced starch accumulation in soybean and Sorghum species. The application of blue light sources is a means of studying the regulation of photosynthetic carbon metabolism in relation to plant growth (Britz and Sager 1990). An increase in the contents of soluble sugar and

starch were observed under CRB-LED treatments in grape (Heo et al. 2006). The present study, which is consistent with previous studies, revealed that the starch concentration is greatest in shoots grown under CRB-LED. The obtained results by Lin et al. (2013) explained the significant increase in total content of soluble sugar in lettuce leaves grown under LEDs (with a high share of red light) than in those grown under fluorescent lamps.

Regarding free amino acid production, it is regulated by various a biotic and biotic environmental factors and depend on how those factors affect photosynthesis and growth (Estrada et al. 1999). And they will be high to prevent cellular oxidative damage (Moyer et al. 2002). Amino acids are the constituents of proteins and play an important role in plant metabolism and development. Herein, light type plays a pivotal role in regulating of the growth, differentiation and metabolism. Results of enzyme activity were similar to those reported by Al-Mayahi (2014), who confirmed that the acceleration of peroxidase activity was associated with increased number of buds in date palm cv. Hillawi. This result is in accordance with earlier report of an enhancement of peroxidase activity in response to incubation shoots under blue or red LED light as compared with fluorescent lights (Manivannan et al. 2015). Hence, the blue-containing LED radiation had positive effects on the action of antioxidant defense mechanisms in tomato seedlings (Kim et al. 2013). The acceleration of enzyme activity was associated with formation of more shoots (Kapchina-Toteva et al. 2005), since plant peroxidases are involved in many functions such as growth and vegetative development, Sharifi and Ebrahimzadeh (2010), and Mamaghani et al. (2010) reported that antioxidant enzymes play an important role in the organogenesis of 20 different plants.

Improvements in LED lighting technologies have increased literary contributions to the scientific database on the impacts of narrow-band wavelength on plant physiology. The results described here clearly showed a relationship between CRB-LED and responses within metabolic pathways in shoots of date palm.

Applications of CRB-LED resulted in significant increases in mineral elements in the shoots, most notably increases in K, Mg and Na.

The type of lamps emitting may have a significant effect on nutrient status of tissues. It can be concluded that the chemical composition of shoots was varied according to the type of light.

Results proved that the differential synthesis of proteins is associated with the growth of shoots under CRB-LED and white fluorescent lights (control treatment). To relate growth patterns precisely to the accumulation and synthesis of gene products, the extant and newly synthesized. The flexibility in fabrication of mixed LED panels with different peak emissions in one plant growth chamber may also provide a desirable spectral emission to achieve a determined growth or physiological response. Which, indicates that plant development and physiology is strongly influenced by blue or red light (Olle and Virsile 2013).

Conclusion

CRB-LED used in the present study was proven to be a promising light source in comparison with the fluorescent lights sources. Our results suggests that CRB-LED enhanced organogenesis of date palm tissues. Additionally, CRB-LED allows a substantial increase of soluble carbohydrate, starch and free amino acids in shoots, hence, the shoots were found to have higher contents of potassium, magnesium and sodium when they were grown under CRB-LED compared with fluorescent lights. Furthermore, results suggests; that the CRB-LED induced the synthesis of newly protein bands with 38 and 60 kDa than those shoots growing under white fluorescent lights (control treatment).

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