The use of blue-green algae in increasing the efficiency of the tissue culture system in date palm *Phoenix dactylifera* L. cv. "Barhee"

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Abstract. The present study was carried out in the Plant Tissue Culture Laboratory, Basrah, Iraq for the period 2015-2017. The aim of the study was to add the biomass extracts of *Oscillatoria tenuis* to the media for in vitro culture of date palm (*Phoenix dactylifera* L.) cv. Barhee to determine its effect on shoot proliferation. The MS medium supplemented with 20% of the biomass water extract of blue-green algae resulted in a significant increase in the percentage of response to proliferation and rooting of the shoots. The same treatment resulted in an increase in the number of shoots producing from proliferation, primary adventitious roots, total chlorophyll content, nitrogen, phosphorus and potassium in leaf. The treatment control recorded the lowest values in all the traits mentioned above. The results of the study showed that the irrigation treatment of 20% of the biomass water extract of blue-green algae resulted in a significant increase in the plant survivor rate, plant height and the number of leaves of the acclimatized plants; while the control gave the lowest values in all the traits mentioned above.
Key Words: biomass extract, micropropagation, MS salts, *Oscillatoria tenuis*, proliferation.

Introduction. Dates palm (*Phoenix dactylifera* L.) trees are fruit trees that are economically important to humans and animals because their fruits are of high nutritional value (Al-Baker 1972). There are more than 600 cultivated varieties in Iraq. The "Barhee" variety is one of the cultivars and best commercial varieties cultivated in Iraq, which the farmer wishes to cultivate and propagate his high economic income for the quality of fruit and the consumer's request to buy it. The traditional method of propagating *P. dactylifera* in Iraq is by offshoots. "Barhee" cultivar is one of *P. dactylifera* varieties that produce a small number of offshoots and the cost of purchasing them is high. Many researchers resorted to the use of micropropagation technique to overcome these problems (Muter 1991). The success of plant tissue culture depends primarily on the type of medium and growth regulators used. Therefore, the use of blue-green algae in the culturing system may contribute to overcoming some of the obstacles that affect the micropropagation process. Algae may be used as substitutes for some expensive chemicals, such as vitamins and antibiotics, which are added to the medium prepared for in vitro culture (Zaccro et al 2006; Banerjee & Sharivastava 2008). Blue-green algae may produce various bioactive substances, including growth regulators, which can be used in the propagation of vegetables, fruits and cut flowers. These substances include gibberellins, auxins, cytokinins, jasmonic acid, ethylene and abscisic acid (Molnar & Ordog 2005; Gupta & Agarwal 1973; Strik et al 2002). Researchers have found that these algae have the ability to produce, release and accumulate these substances from their cells (Keerthiga et al 2012). Those blue-green algae were used as an alternative to MS salts in the micropropagation of the Brahmi (*Bacopa monnieri* L.) plant. Shoots were formed and grown in culture in the medium, which was supplied with blue-green algae, sucrose and kinetin by 2 mg L^{-1} (Mehta et al 2012). Keerthiga et al (2012) found that the nodal segment explant of *Wedelia trilobata* plant was best regenerated when cultured in the medium with MS salts and biomass water extract (BWE) for blue-green algae when compared to control treatment (without BWE). The researchers concluded that the

addition of these extracts is much better than the addition of industrial growth regulators to the medium of stimulating the growth of vegetative shoots (Keerthiga et al 2012; Mehta et al 2012). The objective of this study was to investigate the role of adding the biomass water extract of blue-green algae in the media prepared for the stages of micropropagation of *P. dactylifera* trees cv. "Barhee".

Material and Method

Preparation of explant and direct organogenesis. The study was conducted in the Plant Tissue Culture Laboratory of Basrah in the area of Bahadriya, district of Abu al-Khassib, the province of Basrah in the 2015-2017 period. *P. dactylifera* offshoots of cv. "Barhee" were obtained from aged 3-4 years from one of the *P. dactylifera* orchards of Abu Al-Khassib for use as a source of explant plant parts. The offshoots were sliced to the shoot tips (Figure 1A).

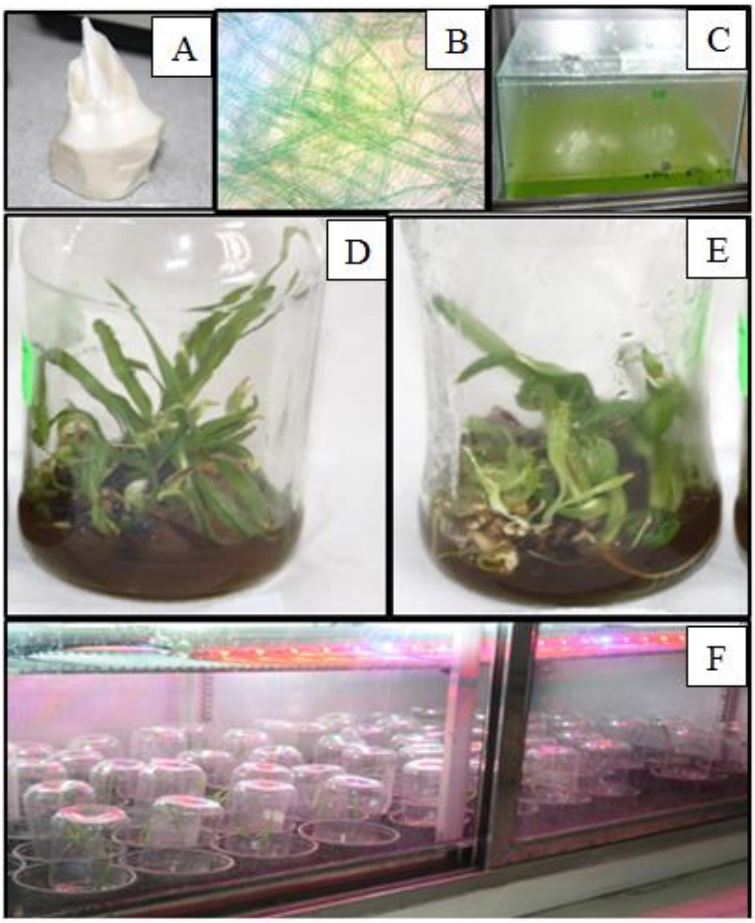

Figure 1. Micropropagation of *Phoenix dactylifera* cv. "Barhee": A - Shoot tip; B - Blue-green algae (*Oscillatoria tenuis*); C - Culture basin of blue-green algae; D - Shoot proliferation in MS medium supplemented with 20% BWE of algae; E - Shoot proliferation in MS medium supplemented with 10% BWE of algae; F - Acclimatization of *P. dactylifera* plants.

After that, the shoot tips were placed for 24 hours in refrigerator in antioxidant solution consisting of citric and ascorbic acid and distilled water at a concentration of 150 and 100 mg L^{-1}, respectively. The following day, the shoot tips were sterilized for 60 min with mercury chloride ($HgCl_2$) sterilized solution at a concentration of 0.001% with the addition of several droplets of Polysorbate 20 to increase the efficiency of sterilization of the outer surfaces of explants. The shoot tips were then grown in the MS medium (Murashige & Skoog 1962). Some chemicals indicated in Table 1 and growth regulators

were added to the MS medium at a concentration of 0.2 mg L^{-1} of BA, 0.2 mg L^{-1} of 2ip, 0.2 mg L^{-1} of Kinetin and 6.0 mg L^{-1} of NAA and 60 g L^{-1} of sucrose. Direct adventitious bud initiation was induced after eight weeks of in vitro culture.

Table 1

Chemical components of the MS media prepared for the proliferation of *Phoenix dactylifera* cv. "Barhee"

Components	*Quantity (mg L^{-1})*
MS salts	4330.0
$Na_2H_2PO_4.2H_2O$	185.0
D-Pantothenate calcium (Vit. B_5Ca)	20.0
Adenine sulfate	80.0
Calcium nitrate tetrahydrate	2000.0
Inositol	100.0
Thiamin	0.04
Polyvinylpyrrolidone 10	500.0
L-Glutamine	200.0

Cultivation and development of algae (Oscillatoria tenuis). *O. tenuis* (Figure 1B) was grown in sterile glass basins of 30 x 50 x 40 cm (Figure 1C), containing the sterile BG11 liquid medium. These cultures were then placed in a growth chamber at a temperature of 25±2°C and under the light intensity of 60 µmol m^{-2} s^{-1}. These blue-green algae were developed using a fluorescent light of 24 hours light. The cultures have been continuously shaken for the purpose of obtaining a growing mass of algae. The date of the algae harvest was in the Stationary Phase (McKinney 1941). The BWE of algae was extracted by taking 5 g of algae biomass after it was filtered by through Whatman No. 1 papers (Medina-Jaritz et al 2011).

The water extracts of the biomass were added to the media prepared for the two stages of shoot proliferation and rooting according to the following treatments:

1. Control treatment: Shoots of *P. dactylifera* were cultured on the full-strength MS medium supplemented with other chemicals (Table 1).
2. 10% BWE treatment: Shoots of *P. dactylifera* were cultured on the full-strength MS medium supplemented with 10% concentration of biomass water extract (BWE) of algae and other chemicals (Table 1).
3. 20% BWE treatment: Shoots of *P. dactylifera* were cultured on the full-strength MS medium supplemented with 20% concentration of biomass water extract (BWE) of algae and other chemicals (Table 1).

Also 50 g L^{-1} sucrose, 0.1 mg L^{-1} for each of the 2ip, kinetin and BA, and 6.5 g L^{-1} agar was added to MS medium of shoot proliferation stage. While 40 g L^{-1} sucrose, 0.5 mg L^{-1} NAA and 6.7 g L^{-1} agar was added to MS medium of rooting stage.

After the formation of the plantlets resulting from shoot proliferation of *P. dactylifera* cv. "Barhee" and reached the age of eight weeks after rooting, there were acclimatized after transfer from culture containers and washed with sterilized distilled water to remove the residual effect of the medium. After that, the plantlets were immersed for 5 minutes with the Hymazol 0.5 mg L^{-1} with 10 mg L^{-1} of the tetracycline antibiotic with several drops of the Polysorbate 20 to reduce the surface tension and improve the sterilization efficiency. The plants were then covered with plastic cups to maintain the surrounding humidity (Figure 1F). After that, the plants were irrigated with the following treatments:

1. Control treatment: The plants were watered with distilled water only.
2. Treatment of 10% of the BWE: The plants were irrigated with a biomass extract of blue-green algae at a concentration of 10%.
3. Treatment of 20% of BWE: The plants were irrigated with a biomass extract of blue-green algae at a concentration of 20%.

Studied characteristics:

1. Percentage of response to the shoot proliferation;
2. Number of formed shoots/explant;
3. Shoot length (cm);
4. Total chlorophyll content in shoot leaves: Total chlorophyll was estimated in mg g^{-1} as described in Goodwin (1976);
5. The content of leaves of nitrogen, phosphorus and potassium: The dry samples of the leaves of the shoots were digested according to the method described by Cresser & Parsons (1979). Total nitrogen concentration in leaves (%) was estimated using Micro Kjeldahl. The content of the phosphorus in leaves of the shoot was estimated at g kg^{-1}. The amount of potassium in the leaves of the shoots was estimated in mg kg^{-1} unit according to the methods described by Page et al (1982).
6. Percentage of shoot response to rooting;
7. Number of primary adventitious roots per shoot;
8. The survival success rate was calculated after eight weeks of culturing;
9. Plant height: Plant height was measured in cm units after eight weeks of acclimatization;
10. The number of leaves: The number of leaves per plant was calculated after eight weeks of acclimatization.

Experimental design and statistical analysis. All experiments for the current study were designed according to Complete Randomized Design (CRD). Each treatment in the experiments under study was repeated five times. The data were analyzed statistically using the analysis of variance. Compare the mean of treatments using Revised Least Significant Difference (R-LSD) at a 5% probability level based on Al-Rawi & Khalaf Allah (2000).

Results and Discussion. Table 2 shows the significant superiority of the medium containing 20% of the biomass water extract (BWE) of blue-green algae in the percentage of response to the shoot proliferation, the number of shoots/explant, shoot length and the total chlorophyll content in the leaves compared to the other two treatments (Figure 1D & E). As the treatment 20% of the BWE recorded 90.00%, 60.75 shoots/explant, 4.78 cm and 0.393 mg g^{-1} fresh weight respectively. While the control treatment (without BWE) recorded the lowest values in the percentage of response to the shoot proliferation, the number of shoots/explant, shoot length and the total chlorophyll content of 81.50%, 50.75 shoots/explant, 4.18 cm and 0.323 mg g^{-1} fresh weight respectively.

Table 2

Effect of adding the biomass water extract (BWE) of blue-green algae to the MS medium in some vegetative characteristics of *Phoenix dactylifera* shoots

Treatment (%)	*Response to proliferation (%)*	*No. of shoots/explant*	*Shoot length (cm)*	*Total chlorophyll (mg g^{-1} DW)*
Control	81.5	50.75	4.18	0.323
10% BWE	87.5	55.75	4.53	0.345
20% BWE	90.0	60.75	4.78	0.393
R-LSD (P≤0.05)	1.02	2.52	0.051	0.012

Table 3 shows that the MS medium supplemented with 20% of BWE was significantly different in the percentage of total nitrogen and the phosphorus and potassium content in the leaves compared to the 10% of BWE and control treatments. This treatment gave the highest values of leaf content of nitrogen, phosphorus and potassium which reached 3.250%, 8.469 g kg^{-1} and 0.42 mg kg^{-1} respectively. While the control treatment recorded the lowest values in the percentage of total nitrogen, phosphorus and potassium content of 2.942%, 7.962 g kg^{-1} and 0.145 mg kg^{-1}, respectively.

Table 3

Effect of adding the biomass water extract of blue-green algae to the MS medium in some minerals content of *Phoenix dactylifera* shoots

Treatment (%)	*Nitrogen (%)*	*Phosphorus (g kg^{-1})*	*Potassium (mg kg^{-1})*
Control	2.942	7.962	0.145
10% BWE	3.029	8.099	0.275
20% BWE	3.250	8.469	0.423
R-LSD (P≤0.05)	0.063	0.140	0.056

The results in Table 4 show that the medium supplemented with 20% of BWE of algae gave the highest significant difference in the percentage of the response of the shoots to the rooting and the number of primary adventitious roots compared with the other two treatments. This treatment recorded the percentage of response to rooting of the shoots and the number of formed primary adventitious roots 88.75% and 3.5 roots/shoot, respectively. While the control treatment recorded the lowest values in the percentage of the response of the shoot to rooting and number of primary adventitious roots of 80.00% and 2.00 roots/explant, respectively.

Table 4

Effect of adding the biomass water extract of blue-green algae to the MS medium in % rooting response and the number of roots per shoot of *Phoenix dactylifera*

Treatment (%)	*% response to rooting*	*No. of roots/shoot*
Control	80.00	2.0
10% BWE	82.50	2.5
20% BWE	88.75	3.5
R-LSD (P≤0.05)	1.12	0.102

The results in Table 5 shows that the acclimatized plants that were irrigated with the biomass water extract of blue-green algae of 20% concentration, exhibited significantly superior survival percentage, plant height and number of leaves compared with the other two treatments after eight weeks of culture. This treatment recorded 85.00%, 16.20 cm and 2.00 leaves/plant, respectively. While the treatment of watering with distilled water gave the lowest values in the qualities referred to above as 65.20%, 11.25 cm and 1.00 leaf/plant, respectively.

Table 5

Effect of irrigation with biomass water extract of blue-green algae in some growth characteristics of acclimatized *Phoenix dactylifera* plants

Treatment (%)	*Survival (%)*	*Plant height (cm)*	*No. of leaves per plant*
Control	65.2	11.25	1.00
10% BWE	82.5	14.20	1.80
20% BWE	85.0	16.20	2.00
R-LSD (P≤0.05)	2.54	0.201	0.68

The improvement in vegetative and root growth in *P. dactylifera* growing by in vitro culture is due to the role of biomass water extract added to the MS medium. Blue-green algae produce various bioactive substances, including growth regulators, which can be used in the production of vegetables, fruits and cut flower plants. These substances include gibberellins, auxins, cytokinins, ethylene and abscisic acid. It was found that these algae have the ability to produce jasmonic acid (Strik et al 2002; Molnar & Ordog 2005). The researchers also found that these algae have the ability to produce, release

and accumulate these substances from their cells (Keerthiga et al 2012). The results of the current study were similar to the results obtained by other researchers in their studies on other plants such as *B. monnieri* (Mehta et al 2012) and *W. trilobata* (Keerthiga et al 2012). The increase in total chlorophyll content in the leaves is due to the increased concentration of nitrogen in the MS medium as a result of the addition of algae biomass extract. These results were agreed upon by Meziani et al (2016). Algae extract contains positive calcium ions (Ca^{+2}) and is associated with the increasing effect on plant growth as it regulates the metabolic and vital processes in the plant cell (Shariatmadari et al 2015). This explains the increase in the amount of nitrogen; phosphorus and potassium in the shoot of plants growing on the medium containing the biomass water extract of algae. The addition of the 20% concentration of the biomass water extract from the blue-green algae to the MS medium prepared to proliferation and rooting of *P. dactylifera* was optimal due to the interaction between the active biomaterials in the plant tissues and those found in the algae biomass extract, which contributed to improved cell division and growth (Zaccaro et al 2006; Banerjee & Sharivastava 2008; Seema et al 2011). The results of the present study are in contrast with the results obtained by Mehta et al (2012). They showed that the concentration of 10% of the biomass extract of the blue-green algae (*Phormidium subincrustatum*), which was added to MS medium, gave the best results compared to the control treatment (without biomass extract).

The reason for increasing the percentage of the success of acclimatization is due to its treatment with the biomass water extract of the algae with a concentration of 20%, which may be due to plant hormone-like substances produced by algae. That its presence contributed to the continued growth of acclimatized plants by stimulating and encouraging their meristematic tissues to grow. The role of abscisic acid, which is one of the components of biomass extract, has contributed to the improvement of root growth and control of the closure of the stomata, which has had a positive effect in preserving the water content of the acclimatized plants.

Conclusions. The addition of the biomass water extract (20%) of the blue-green algae (*O. tenuis*) to the MS medium prepared for micropropagation of *P. dactylifera* contributes to improving vegetative and roots growth and increases the percentage of acclimatization success. As well as the possibility of using the biomass extract of algae as a promoter component of the MS medium prepared for in vitro culture.

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