The effect of adding extracellular products of blue-green algae to the MS medium on the callus, shoot, and root induction of the ber tree, Zaytony cultivar

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Abstract. The investigation was achieved in the plant micropropagation laboratory of the Fadeck Company Agriculture in the Al-Bahadriya province, Abul-Khaseeb District, Basrah, Iraq, in 2023. This study was achieved to determine the role of enriching the nutrient medium by an extracellular product of the algae *Oscillatoria tenuis* in the callus induction, indirect shoot regeneration, and root initiation of ber (*Ziziphus mauritiana* Lam. cv. Zaytoni) trees in vitro by using shoot tips as explants. The data revealed that the full-strength MS + CEP medium obtained an excellent shoot tip response to the callus initiation and the fresh and dry weight of the callus contrasted to the effect of other media. It recorded the highest response in terms of callus induction and values of the fresh and dry weight of the callus attaining 90%, 480 mg, and 106 mg, respectively. The callus induced in the treatment with full-strength MS + CEP medium was friable and dark green. The half-strength MS + CEP treatment induced a light green callus. The full-strength MS + CEP treatment was significantly superior in terms of callus response to indirect shoot regeneration and the number of shoots contrasted to the other media, attaining 90% and 7.20 shoots per culture, respectively. The MS half-strength + CEP medium was significantly better in terms of shoot response to root formation and the number of roots contrasting to the other media, which registered 40% and 3.2 roots per shoot, respectively.
Key Words: Cyanobacteria extracellular products (CEP), explant, MS salts, shoot regeneration.

Introduction. The ber tree belongs to the family Rhmanaceae and the genus Ziziphus, which contains more than 100 species of plants that include trees and shrubs with evergreen or deciduous leaves. The trees of this species inhabit the temperate, tropical, and subtropical regions of the world (Pareek 2001). These tree fruits are of high nutritional value due to their high content of vitamin C and are also rich in carotenoids. This tree has multiple uses, as its leaves, roots, and bark are used as a source of medical treatment and animal fodder (Yamdagni et al 1985; Morton 1987; Abbas 1997). The ber plant has received great attention on a global level as an unexploited fruit tree with a great future. The common method of propagation for ber trees is grafting or budding onto seed rootstocks, which is the most widely, used method for vegetative propagation due to the difficulty of forming adventitious roots (Pareek 2001). The method of propagation by grafting or budding is not suitable for commercial production. This is due to the long period it takes to reach the fruiting stage of trees and to obtain small numbers of plants produced from this method. In addition, it depends on the appropriate period for this method of reproduction (Sudhersan et al 2001; Assareh & Sardabi 2005; Assareh et al 2005). There are several attempts made on the micropropagation of ber trees with the aim of overcoming the problems aforementioned when using traditional asexual propagation methods. More numerous new plants identical to the mother plant and bearing early fruit are obtained without being bound by a suitable growing season for propagation (Rathore et al 1992; Hossain et al 2003; Sudhersan & Hussain 2003; Assareh & Sardabi 2005). The success of the propagation technique through tissue culture depends primarily on the organic and inorganic components of the nutrient

medium and the type of growth regulators added to it. Therefore, adding extracellular algae products to the nutrient medium prepared for tissue propagation may help to overcome some of the obstructions that influence micropropagation.

Cyanobacteria may replace the use of some expensive chemicals, such as vitamins, antibiotics, and growth regulators (Zaccaro et al 2006; Banerjee & Sharivastava 2008). Cyanobacteria may create different bioactive compounds, involving growth regulators, which can be used in the production of fruits, vegetables, and cut flowers. Among these compounds are auxins, gibberellins, cytokinins, ethylene, and abscisic acid. It has been found in studies that these algae can produce jasmonic acid (Gupta et al 1973; Stirk et al 2002; Molnar & Ordog 2005). In another study, investigators revealed that cyanobacteria can create these substances in their cells, after which they are released and accumulated (Keerthiga et al 2012). This investigation was achieved to define the role of enriching the nutrient medium by an extracellular product of the algae *Oscillatoria tenuis* in the callus induction, indirect shoot regeneration, and root initiation of ber (*Ziziphus mauritiana* Lam. cv. Zaytoni) trees in vitro, by utilizing shoot tip explants.

Material and Method. The investigation was achieved in the Plant Micropropagation Laboratory of the Fadeck Company Agriculture in the Al-Bahadriya Province, Abul-Khaseeb District, Basrah, Iraq, in 2023. 6-7 years old ber trees of the Zaytoni cultivar were obtained from a ber tree orchard in Abul-Khaseeib District to be used as a source of shoot tip explants. Shoot tips were excised from branches of ber trees as Zaytoni cultivar (1 cm length). After that, the tips of the shoots were put in the refrigerator for 24 h in an antioxidant solution containing ascorbic acid, citric acid, and sterile water distilled at concentrations of 100 and 150 mg L^{-1}, respectively. Then, after one day, these shoot tips were sterilized for one hour with a sterile solution consisting of mercury chloride ($HgCl_2$) at a concentration of 0.001%; several drops of Tween 20 were added to increase the effectiveness of sterilizing the external surfaces of the explants against the microorganisms causing the contamination. After that, the shoot tip explants were cultured in an MS medium (Murashige & Skoog 1962). Several organic and inorganic compounds and growth regulators were added to enrich the MS medium; 40 mg L^{-1} adenine sulfate, 185.5 mg L^{-1} of $Na_2H_2PO_4.2H_2O$, 60 mg L^{-1} of inositol, 10 mg L^{-1} of D-pantothenate calcium, 1 mg L^{-1} of Thiamine, 100 mg L^{-1} of Glutamine, 30 g L^{-1} of Sucrose, 1 g L^{-1} of Polyvinyl pyrrolidone, 5 mg L^{-1} of BA, 0.1 mg L^{-1} of NAA, 7 g L^{-1} of Agar. Callus formation was initiated after 45 days of in vitro culturing.

Culturing and growing of blue-green algae. *O. tenuis* was produced in sterile glass containers of 500 cm^3 volume, including the sterile liquid of BG11 medium. These cultures were then put at the temperature of 25±2°C in a growth room and under the light intensity of 60 µmol m^{-2} s^{-1}. These cyanobacteria were generated by exposure to 24-hour light using a white fluorescent light. The culture vessels have been subjected to constant vibrations in order to boost the growth of cyanobacteria. The algae harvest date was during the Stationary Phase (Mackinney 1941). Cyanobacteria Extracellular Products (CEP) were isolated by taking 5 g of lyophilized algae, then 100ml of distilled water were added and the mixture was placed on a magnetic stirrer hotplate at 100°C for 20 minutes. The mixture was then cooled and filtered using filter papers, of the Whatmann No. 1 type. The filtrate was kept in the refrigerator (4°C) until usage. The CEP extracts were supplied to the two experiments of the callus induction, indirect shoot regeneration, and root initiation according to the subsequent combinations:

1. Control treatment: Full strength of MS salts + distilled water (CEP0).
2. Full strength of MS salts + CEP extract (CEP1).
3. Half strength of MS salts + CEP extract (CEP2).
4. Quarter strength of MS salts + CEP extract (CEP3).

The plant growth regulator combinations enriched to the MS media in the indirect shoot regeneration and rooting stages was changed to (2 mg L^{-1} BA + 0.2 mg L^{-1} NAA) and (0.1 mg L^{-1} BA + 10 mg L^{-1} IBA), respectively.

The growth and development indicators.

1. Response to callus induction (%).
2. Fresh and dry weight of callus (mg).
3. Response to indirect shoot regeneration (%).
4. Number of indirect shoots (shoots per culture).
5. Shoot length (cm).
6. Response to root initiation (%).
7. Number of roots (roots per shoot).
8. Root length (cm).

Statistical analysis of data. The simple investigations were planned according to a complete randomized design (CRD). Each treatment was replicated 10 times. The data were statistically examined utilizing the analysis of variance (ANOVA). The means of the treatments were compared utilizing the revised least significant difference test (R-LSD test) at the 1% probability level, according to Al-Rawi & Khalfallah (2000).

Results and Discussion

Callus induction. The data of Table 1 indicate that the full-strength MS medium + CEP was significantly dominant in terms of response of the shoot tips to callus initiation and the fresh and dry weight of the callus contrasted with the response to other media they recorded the highest response in terms of callus induction (the Callus induction rate (%) = (Total number of explants produced callus/ Total number of explants cultured) × 100%) and values of the fresh and dry weight of the callus, attaining 90%, 480 mg, and 106 mg, respectively. The combination of the medium MS, quarter strength + CEP, registered the lowest response in terms of callus induction, and the fresh and dry weight of callus, attaining 40%, 136 mg, and 20 mg, respectively. The callus induced in the treatment with full-strength MS + CEP medium was friable and dark green. The half-strength MS + CEP, induced callus was light green. The callus was a white, compact tissue when treated with the quarter-strength + CEP (Table 1).

Table 1

The effect of cyanobacteria extracellular products on the induction of callus of ber Zaytoni cultivar, six weeks after culturing

Treatment	*Callus induction (%)*	*Fresh weight (mg)*	*Dry weight (mg)*	*Callus color*	*Callus texture*
Control	70%	325	84	Light green	Compact
CEP1	90%	480	106	Dark green	Friable
CEP2	60%	178	32	Light green	Friable
CEP3	40%	136	20	White	Compact
R-LSD (0.01)	4.22	6.44	4.26	-	-

Indirect shoot regeneration. The data from Table 2 indicate that there are significant differences between the treatments in the callus response indicators to indirect shoot regeneration and the number of shoots formed from the callus after eight weeks of culturing. The full-strength MS + CEP treatment was significantly superior in terms of callus response to the indirect shoot regeneration and number of shoots compared to the other treatments, and recorded 90% and 7.20 shoots per culture, respectively. The treatment with the medium quarter-strength MS + CEP recorded the lowest response in terms of indirect shoot regeneration, and number of shoots attaining 10% and 3.20 shoots per culture, respectively. The control treatment recorded the highest shoot length value of 5.16 cm, which differed significantly from the rest of the treatments. The medium treatment, half-strength MS + CEP, recorded the lowest shoot length, which attained 2.60 cm (Table 2).

Table 2

The effect of cyanobacteria extracellular products on the indirect shoot regeneration of ber Zaytoni cultivar, eight weeks after culturing

Treatment	*Regenerated shoot (%)*	*Shoot numbers (shoots per culture)*	*Length of shoot (cm)*
Control	40%	3.40	5.16
CEP1	90%	7.20	3.48
CEP2	80%	5.20	2.60
CEP3	10%	3.20	4.44
R-LSD (0.01)	6.20	0.51	0.12

Root initiation. It is clear from the data in Table 3 that there are significant differences between the treatments in the response of the shoots to the initiation of roots and the number and length of the roots after four weeks of culturing. The medium MS half-strength + CEP was significantly dominant in terms of shoot response to root formation and the root numbers contracted, compared to the other media, registering 40% and 3.2 roots per shoot, respectively. The quarter-strength MS + CEP treatments registered the lowest averages in the shoot response to the root formation and the shoot numbers, attaining 10% and 1.2 roots per shoot, respectively. The data of Table 3 reveal that the control medium registered a significant enhancement in root length compared to the rest of the treatments, which reached 3.50cm, while the full-strength MS medium + CEP registered the lowest root length of 1.80cm.

Table 3

The effect of cyanobacteria extracellular products on the root initiation of ber Zaytoni cultivar, four weeks after culturing

Treatment	*Regenerated root (%)*	*Root numbers (roots per shoot)*	*Length of root (cm)*
Control	20%	1.40	3.50
CEP1	20%	2.40	1.80
CEP2	40%	3.20	2.06
CEP3	10%	1.20	2.84
R-LSD (0.01)	4.28	0.10	0.11

The increase in explant response to the induction of callus, indirect shoot regeneration, and the formation of roots on the shoots when the explants are grown on full-strength MS medium supplemented with CEP, is attributed to the role of CEP in enriching the medium with like-substances to auxins, cytokinins, gibberellins, and jasmonic acid (Stirk et al 2002; Shanab et al 2003; Molnar & Ordog 2005; Ahmed et al 2010). In addition, it may be because blue-green algae can produce many chemical compounds and secondary products of cellular metabolism necessary for growth through their release and accumulation. These substances play an important role in stimulating cellular division, cell enlargement, and organogenesis, and this was proven by a group of researchers through their studies on blue-green algae extracts (Keerthiga et al 2012).

The results of this investigation conformed with the results of other investigations conducted on several plants' in vitro cultures, regarding the addition of extracts of algae extracellular products of various species to the nutrient media. Researchers noted that this addition contributed to improving vegetative and root characteristics during the stages of tissue culture (Shanab et al 2003; Manickavelu et al 2006; Seema et al 2011; Ghasolia et al 2013). The reason for the superiority of the full-strength MS medium supplemented with CEP in the shoot regeneration stage is the plant growth regulators that enriched the MS medium, which interacted with the growth promoters and stimulators in the CEP, encouraging the withdrawal of nutrients, cell division, organ differentiation, and growth, supporting the formation of adventitious shoots and improving their vegetative characteristics (Jazinizadeh et al., 2015). The cause for the

significant elongation in the shoot at the half-strength MS medium including the extract of blue-green algae extracellular substances is attributed to the little competition for nutrients in the medium between the few shoots, which increased their length, which led to an increase in the cellular division and elongation of shoots. Blue-green algae can fix nitrogen by utilizing the ATP energy from the photosynthesis process to ammonia using nitrogenase enzyme (Waterbury et al 1979; Wolk 1980). The reason for the superiority of the half-strength MS medium + CEP in terms of root initiation, formation, and increased root numbers is that the half-strength MS + CEP salts were at optimal concentrations for root induction and growth thus encouraging cell division and differentiation, and consequently the formation of adventitious roots from the bases of the shoots and their growth (Keerthiga et al 2012).

Conclusions. Adding an extract of blue-green algae extracellular products (bioactive compounds, growth regulators, and nutrients) to the MS medium played an important role in enriching the medium. This was reflected positively in stimulating the growing shoot tip explants to callus initiation, indirect shoot formation, and root initiation. Full-strength MS medium supplemented with CEP was the best treatment for inducing callus and forming adventitious shoots of ber (*Ziziphus mauritiana* Lam. cv. Zaytoni).

Conflict of interest. The authors declare no conflict of interest.

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