



Effect of Bacterial Cell Wall Components on Callus Growth of *Phoenix dactylifera* L. cv. Barhee Propagated Through *In vitro*

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Abstract: This study was conducted on shoot tip explants of date palm cv. Barhee to investigate effect of bacterial cell wall components on initial callus growth. Three concentrations of bacterial media (0.5 and 1.0 and 2.5) v/v were used for growing callus by tissue culture technique. MS media was served as a control treatment. Results showed that all bacterial media treatments increased callus fresh weight and nitrogen concentration of media significantly as compared to that of MS control media. Bacterial media treatment of 2.5 (v/v) gave the highest values of callus fresh weight and nitrogen concentration (518 mg and 3.3089%) respectively, whereas MS control media had the lowest values (193 mg and 0.01%) respectively. There was a significant increase in callus fresh weight at 60 days incubation over that of 30 days incubation. The combination of bacterial media treatment at 2.5 (v/v) and incubation period of 60 days recorded the highest significant increase in callus fresh weight (631 mg), whereas the combination of MS control media treatment and incubation period of 30 days had the lowest value (166 mg) in this respect.

Key words: *Phoenix dactylifera* L., Barhee. *In vitro*, bacterial cell wall, initial pro callus, nitrogen.

Introduction

Tissue culture technique of explants in vegetatively propagated plants is an alternative method to obtain rapid clonal multiplication. MS media containing amino acids and vitamins have profound effect on explants propagated via tissue culture technique. Optimization of such compounds can stimulate regeneration in recalcitrant cultivars (Benson, 2000). A new benefit component serves as a nutrition in callus growth. Amino acids are beneficial for embryogenic callus growth

of date palm (Abou El-Nil and Al-Ghamdi, 1986).

The most available nutrition is that has a new and harmless component (Abd El-Zaher, 2008). Many studies were made to compare and provide the most beneficial nutrition to embryogenic callus growth in which new components were isolated from bacteria that contains many natural chemicals, such as, amino acids, glycoproteins, lipoproteins, sugars and some acids, such as, acetyl muramic acid,

many other chemicals and some undiscovered materials (El-Din *et al.*, 2007).

In the last forty years, sergeants dealt with information that has become available about the general structure of the bacterial cell wall. Mucopolysaccharide amino acid (e.g. alanine, glutamic acid, glycine, LL-DAP and Meso-DAP) and sugars (e.g. galactose, glucose, mannose and rhamnose) were found in bacterial cell wall components (Cummins, 1970; El-Din *et al.*, 2007). Sugars as carbon source, urea and amino acids as organic nitrogen source are very important components in the processes of plant propagation which can be added to media in limited concentrations (Steinhart *et al.*, 1961; Behrend and Mateles, 1975; Joseph, 1976; Abdel-Rahim *et al.*, 1998; Osman, 2010). The amino acid glutamine is beneficial for embryogenesis and embryogenic callus growth of date palm (Abou El-Nil and Al-Ghamdi, 1986).

Thiamine is generally considered to be an essential ingredient for plant tissue culture (Bhojwani and Razdan, 1983). Also, Al-Khayri (2001) showed that MS medium supplied with 0.5 mg/L thiamine and 2.0 mg/l biotin recorded the highest callus growth of date palm as compared to that of MS medium only. Moreover, Khlifi and Tremblay (1995) reported that this media can be used for conifers as well as other plant species and L-glutamine can be considered as a major component of the medium during maturation of somatic embryo.

The present study was carried out to investigate effect of bacterial cell wall components that contain organic nitrogen, supplemented as amino acids and sugars, on callus initiation and promotion of callus growth of date palm cv. Barhee using *in vitro* propagation technique.

Materials and Methods

Apical buds of Barhee date palm cultivar were isolated and used for callus growth. Callus was cultured on two types of media, the first was MS media and the second was

media containing bacterial cell wall components only. The concentration and composition of MS media was made according to the method of Murashige and Skoog, (1962). Solidification of MS medium was achieved by adding 0.7 % sugar and then adjusted to pH 5.8 before autoclaving. MS medium was served as a control treatment.

Bacterial media was used with three concentrations in this experiment. Bacterial media were considered as promoting media treatments. The prepared MS and bacterial media were poured into heated sterilized 50 ml glass tubes and 1 cm length explants, taken from apical buds, were cultured on both media. Each treatment was replicated ten times and the average of callus fresh weight, in replicated tubes, was used for calculation.

Bacterial component media

The bacteria (mixed of Gram positive Cocci *Staphylococcus* spp. and Gram-positive *Bacilli bacillus* spp.) were grown in a peptone–yeast–extract–glucose medium, containing also cysteine (0.06%) and bicarbonate (0.5%). The mixture was digested with pronase for 2 hrs. at 56 °C and shaken for 120 minutes. The solution was tested microscopically and by culture to detect no living bacteria was found. The whole mixture was used as a nutrition media. The bacterial media concentrations were made of 0.5ml and 1.0 ml and 2.5 ml cell wall components per 10 ml double distilled sterile water. These media treatments were supplied with 0.6 gm agar for solidification. These bacterial media were autoclaved as in MS control media. Extraction of nitrogen in bacterial media was achieved using the process of Cresser and Parsons (1979) and nitrogen concentration was estimated by Distillation Unit Apparatus according to the method described by Page *et al.* (1982).

The experiment was terminated after incubation period of 60 days and readings of callus fresh weight were taken twice at

(30 and 60) days of incubation to obtain the percentage of callus growth increase. Data of callus growth, as expressed by callus fresh weight, were analyzed following the method of analysis of variance for completely randomized design and the least significant difference test at $p \leq 0.05$ level was used to determine significant differences between treatment means.

Results and Discussion

The obtained data by both bacterial and MS control media of Barhee callus fresh weight were presented in Table 1. Initial callus growth of both media were shown in Plates 1 and 2. It was found that all bacterial media treatments increased callus fresh weight significantly in comparison to that of MS control media. There were also significant differences between bacterial media treatments in this respect. The

highest value of callus fresh weight (518.00 mg) was resulted from bacterial media treatment of 2.5 (v/v), whereas the lowest value (193.00 mg) was recorded for MS control media treatment. Regarding the percentage of callus fresh weight, it was clear that all bacterial media concentrations stimulated callus growth, recording higher percentage of 8.03%, 58.55% and 168.39% respectively over that of MS control media. The incubation period of 60 days gave the highest significant value of callus fresh weight (372.00 mg) as compared with that of thirty days incubation (Table 1), with the percentage of callus growth reaching a value of 54.52%. The combination of bacterial media treatments and incubation periods of 30 and 60 days recorded significantly higher callus fresh weight than those of MS control media at both incubation periods (Table 1).

Table (1): Effect of MS control and bacterial media on callus fresh weight (mg) after incubation periods of (30 and 60) days.

Type of media	Incubation periods (day)		Average of media
	30	60	
MS media	166	220	193.00
0.5 Bacterial media (v/v)	180	237	208.50
1.0 Bacterial media (v/v)	212	400	306.00
2.5 Bacterial media (v/v)	405	631	518.00
Average of incubation	240.75	372.00	
LSD at $p \leq 0.05$	Incubation 1.731	Media 2.448	Combination 3.462

Table (2): Nitrogen concentration (%) of MS control and bacterial media.

Type of media	Nitrogen concentration (%)
MS media	0.0100
0.5 Bacterial media (v/v)	1.9600
1.0 Bacterial media (v/v)	2.7400
2.5 Bacterial media (v/v)	3.3089
LSD at $p \leq 0.05$	0.0326

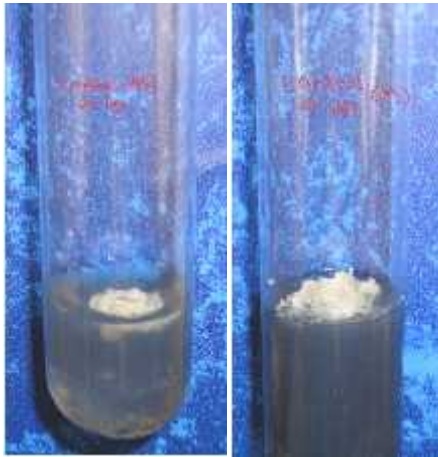


Plate (1): Initial callus growth of MS control media after incubation periods of 30 days (left) and 60 days (right).

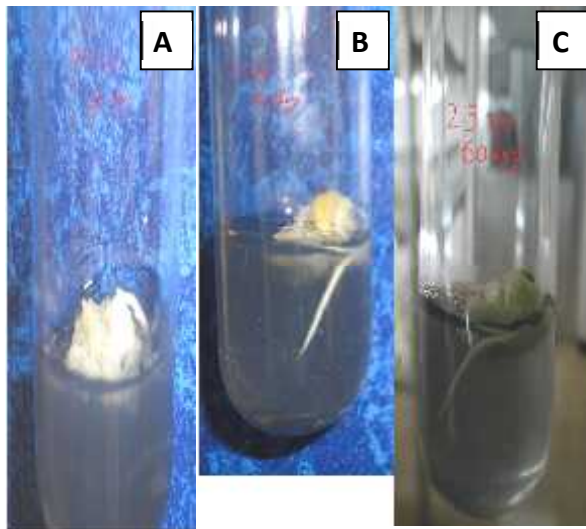


Plate (2): Initial callus growth of bacterial media treatments after incubation periods of 30 and 60 days (A: 0.5 v/v (30 days), B: 1.0 v/v (60 days), C: 2.5 v/v (60 days)).

There were also gradual increases in callus fresh weight as the concentration of bacterial media and incubation period increased.

The highest value of callus fresh weight (631 mg) was recorded for the combination of 2.5 v/v bacterial media treatment and the incubation period of 60 days, whereas the lowest value (166 mg) was produced by the combination of MS control media

treatment and the incubation period of 30 days (Table 1). In regard to MS control treatment, the incubation period of 60 days significantly gave the highest callus fresh weight reaching 220mg as compared to the incubation period of 30 days which recorded 166 mg (Plate 1). A sign of organogenesis was observed in the growing callus of bacterial media treatment of 1.0 (v/v) as induced after incubation period of 60 days (Plate 2), suggesting further investigation in this respect. The higher increases of callus fresh weight of bacterial media over that of MS control media at both incubation periods could be due to the organic compounds of bacterial cell walls which are rich in amino acids: alanine, glutamine, glycine and thiamine, as well as, sugars: glucose, galactose, mannose and rhamnose (Cummins, 1970; El-Din *et al.*, 2007; Olrichs, 2010; Osman, 2010), thereby stimulating the growth of callus and its fresh weight. In addition, these findings were a reflection of bacterial media being free of infection and antibiotics as compared to MS control media. These differences between both media may be due to some enzymes and specific acids that were found in bacterial media, such as, muramic acid (El-Din *et al.*, 2007; Hediger *et al.*, 2013). Our findings were in agreement with those of Mohamed (1996) who found that the application of a single or mixture of amino acids can be used for callus growth promotion and to facilitate plant regeneration in fenugreek, as cultured cells normally capable of synthesizing all the required amino acids.

Data of nitrogen concentration of bacterial and MS control media were shown in Table 2. All the bacterial media had significant increases in nitrogen concentration as compared to that of MS control media, with the highest value (3.3089 %) being given by bacterial media of 2.5 v/v, whereas the lowest value of

nitrogen (0.010%) was recorded for MS control media.

These findings were in line with those of Cummins (1970) and Osman (2010) as they found that bacterial cell walls contained elements, such as, nitrogen, potassium, calcium and copper which may facilitate cell growth. Singh *et al.* (1981) found that nitrogen was the main element for callus growth.

Conclusions

It would seem, therefore, that the enriched bacterial media with nitrogen enhanced cultured cells to synthesize the required nitrogenous compounds, such as, amino acids for the benefit of cell enlargement and growth, resulting in increases of initial callus fresh weight.

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