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RESEARCH ARTICLE

TISSUE CULTURE OF Ficus carica VARIETY BTM-6

Nur Atikah Azhar[a], Zarina Zainuddin[b*]

[a] Department of Biotechnology, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia
[b] Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia
****Corresponding Author Email: zzarina@iium.edu.my***

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ABSTRACT

Ficus carica or commonly known as fig plant is a deciduous plant originated from southwest Asia and eastern Mediterranean. It has many benefits in medical field especially to treat diseases such as rheumatism and haemorrhoids due to its high laxative activity effect. The main objective of this study is to develop *in vitro* clonal propagation method for rapid production of *Ficus carica* variety BTM-6 plantlet using different plant growth regulators (PGRs) through shoot induction and multiplication, rootings and subsequent establishment in soil following acclimatization. Surface sterilisation of the explant was done using sodium hypochlorite as the disinfectant. Pre-treatment of the explants with carbendazim successfully reduced the occurrence of fungal contamination. To investigate the effect of plant growth regulators on shoot induction, explants were cultured in different concentrations of PGRs either singly or in combination. No shoot and root inductions were observed but calli were successfully induced on MS medium containing 2 mg/l BA only, 2 mg/l BA in combination with 0.5 mg/l NAA and MS media supplemented with 0.5 mg/l BA in combination with 0.5 mg/l NAA. A further in-depth study using other different types of plant growth regulators at various concentrations is required in order to establish a complete tissue culture protocol of this particular plant species.

KEYWORDS

Ficus carica, callus, BA, NAA.

1. INTRODUCTION

Ficus carica or commonly called as fig is a deciduous plant from the family of Moraceae and originated from southwest Asia and eastern Mediterranean. The commercial fig is originally from all around the world including the Mediterranean region, Australia, China, Hungary, England and Turkey (Mawa et al., 2013; Qrunfleh et al., 2013). Various forms of figs can be found, either tall and large trees, small trees, bushes or shrubs with extended roots and with simple, alternate, entire or lobate leaves (Soliman et al., 2010). The matured fruit of fig has tough skin and will crack upon ripeness. It has mass-bound seeds with outer ring in white and maroon red jelly-like flesh.

Fig has many benefits to human especially in medical field. Figs are very useful in treating different diseases including hemorrhoids and rheumatism (Paknahad and Sharafi, 2015). Figs are among plant species that have been mentioned in the Holy Quran besides olives, grapes, pomegranates and dates. Few hadiths narrated about the benefits of figs mentioned by Prophet Muhammad (PBUH). Abu ad-Darda' A.S narrated that the Prophet (PBUH) said:

"If I could say that a fruit was sent down from Heaven (to earth), I would say it is figs, because the Heaven's fruit has no stones. Eat it, as it cures hemorrhoids and it is useful for treating gout" (Shahih al-Bukhari)

Therefore, it is obvious that figs are beneficial in Islamic perspectives. Abundant phenolic compounds in the leaf of fig plant has made it beneficial to treat diabetes, hepatic and renal stones. The extract of fig leaves was believed to have anti-diabetic hence reducing glucose level (Ibrahim et al., 2009).

Ficus carica is usually propagated using cuttings, either from softwood cuttings or hardwood cuttings (Sousa et al., 2013). Sexual propagation by using seeds is not preferred because seeds of fig are nonviable (Qrunfleh et al., 2013). Propagation and breeding of woody plants has been widely done using tissue culture technique. Successful case of plantlet regeneration from apical/axillary buds and nodal explants either with or without encapsulation through tissue culture of mulberry (also a member of family Moracae) has been reported (Mustafa and Taha, 2012). Due to the problem of nonviable seeds and also the demand in fig is increasing, it is necessary to find alternative methods for fig propagation, specifically through tissue culture technique that can speed up the rate of propagation

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by using various parts of the plant as the explants with the aid of plant growth regulators for shoot and root induction.

2. Material and Methods

2.1 Mother plant preparation

A healthy growing *Ficus carica* variety BTM-6 was obtained from nursery of Kulliyyah of Science as a mother plant. Healthy shoots with at least 3 nodes were selected for vegetative propagation to ensure the availability of explants for *in vitro* culture. The stems with shoots were cut in slant, moistened with water and dipped in rooting powder prior to inoculation into peat moss in stem propagation cups. The cups were placed under complete shade in a misting room and allowed to grow adventitious roots for two weeks. Water was sprayed on peat moss twice daily.

After two weeks, the plantlets were planted in polybags filled with approximately 5 kg soil. Then, the plants were grown under fully shade in natural growth conditions at Kulliyyah of Science's nursery. Each polybag was watered once daily and fertilized weekly. After approximately 3 months, mother plants were ready for the explants collection for *in vitro* clonal propagation.

2.2 Surface sterilization of explants and culture initiation

Healthy and young leaves were collected from 2-3 months old field grown fig. For surface sterilization, initially, the explants were washed thoroughly under running tap water for overnight. Then, the explants were pre-treated using fungicide (0.2% of Carbendazim) for 30 minutes. After 30 minutes of incubation in fungicide, the explants were rinsed thoroughly using distilled water and finally with sterile distilled. To initiate the surface sterilization steps, the explants were soaked in 70% (v/v) ethanol for 15 seconds. Then, the explants were incubated in 2.5% (v/v) sodium hypoclorite for 15 minutes and lastly, the explants were rinsed using sterile distilled water for three times.

After surface sterilization, the explants were dried using sterilized filter paper. The leaves were cut approximately 1cm x 1cm using sterilized scalpel. Then, the explants were introduced in MS basal medium supplemented with 30 g/l sucrose and 8% agar to evaluate the response of explants on different treatments.

2.3 Shoot induction and multiplication

After surface sterilization, the leave cuttings were cultured in different concentrations of benzyladenine (BA) (0.5, 1.0, 1.5 and 2.0 mg/l) either singly or in combination with 0.5 mg/l kinetin (Kn) or 0.5 mg/l 1-naphthaleneacetic acid (NAA), for shoot induction and multiplication. The MS medium without PGR was used as control. All culture boxes were incubated in a growth room with 25±2°C temperature; humidity 60-70%; 16/8 hours day/light cycle with light intensity of 2500 lx (white luminescence bulb).

3. Results and Discussion

Initially in this study, high level of contamination occurred where fungal contamination was really severe. To overcome the problem with fungal contamination, explants were pre-treated with fungicide. 0.2% carbendazim was used as a pre-treatment. It was observed that with carbendazim pre-treatment, contamination only occurred after few weeks of culturing and the number of contaminated culture boxes were also minimized (Figure 1). Carbendazim is a fungicide that has wide spectrum and has been used to prohibit fungal growth in the production of edible mushroom (Xia et al., 2016). This fungicide is very useful for a wide range of plant diseases, but the effectiveness is limited due to lack of aqueous activity (Leng et al., 2014).

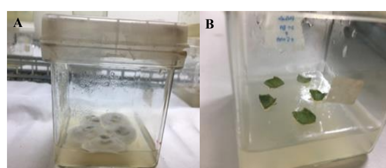

Figure 1: Culture: A) Contaminated with fungi without pre-treatment with carbendazim and B) Clear from contamination after pre-treatment with carbendazim

Results for shoot induction is summarized in Table 1. Instead of producing shoots on all media tested for shoot induction, only formation of calli coud be observed. Calli were successfully induced from treatments of MS medium supplemented with 2.0 mg/l BA, 0.5 mg/l BA + 0.5 mg/l NAA and 2.0 mg/l BA + 0.5 mg/l NAA (Figure 2). Results obtained in this study was in contrast with previous work where the best response of shoot induction and multiplication was obtained on MS medium supplemented with 2.0 mg/l BA + 0.5 mg/l NAA (Howlader et al., 2014). It was also observed that there was no significant difference on the production of shoots among the different varieties. The effectiveness of BA to induce callus formation was also reported by other researchers. For example, callus was found to grow rapidly in saline conditions supplemented with PGR resulted in different genotypic of calli (Benderradji et al., 2011). Embryonic calli could easily formed in medium supplemented with the addition of cytokinin; BAP at low concentration (Bradley et al., 2001). A study on the effect of different types of media and carryover effect on common fig cultivars was conducted. The types of culture media used were Murashige and Skoog (MS), Woody Plant Medium (WPM) and Olive Medium (OM). Calli and shoots were successfully developed on all types of media supplemented with 1 mg/l BA as the PGR (Al-Shomali et al., 2017). Another study on *in vitro* propagation of fig cultivars found that the best proliferation of shoots was on MS media containing 1.0 mg/l BA compared to kinetin. Aboudi cultivar was the best proliferated using cytokinins compared to other cultivars which were Sultani and Conadria (Ahmed-Amen et al., 2014).

Table 1: Response of explants towards different concentrations and combinations of plant growth regulator(s)

Concentration of Plant Growth Regulators	Callus Induction
0 mg/l BA	No
0.5 mg/l BA	No
1.0 mg/l BA	No
1.5 mg/l BA	No
2.0 mg/l BA	Yes
0.5 mg/l BA + 0.5 mg/l NAA	Yes
1.0 mg/l BA + 0.5 mg/l NAA	No
1.5 mg/l BA + 0.5 mg/l NAA	No
2.0 mg/l BA + 0.5 mg/l NAA	Yes
0.5 mg/l BA + 0.5 mg/l Kin	No
1.0 mg/l BA + 0.5 mg/l Kin	No
1.5 mg/l BA + 0.5 mg/l Kin	No
2.0 mg/l BA + 0.5 mg/l Kin	No

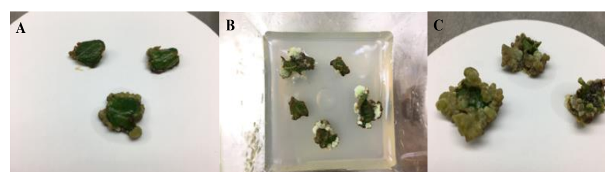

Figure 2: Compact callus induced from leaf explants after 5 weeks of culture on MS medium supplemented with A) 2.0 mg/l BA, B) 0.5 mg/l BA + 0.5 mg/l NAA and C) 2.0 mg/l BA + 0.5 mg/l NAA

4. Conclusion

An optimum protocol for surface sterilization of *F. carica* leave explants has been established to reduce contaminations where the explants were pre-treated with 0.2% carbendazim (fungicide) and further sterilized with 2.5% sodium hypochlorite as the disinfectant. Overall, calli have been successfully induced on MS media supplemented with 2.0 mg/l BA singly and also on MS media supplemented with 0.5 mg/l BA in combination with 0.5 mg/l NAA and 2.0 mg/l BA in combination with 0.5 mg/l NAA.

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