Malaysian Applied Biology (2023) 52(5): 35-40
https://doi.org/10.55230/mabjournal.v52i5.cp19

Research

Optimization of Different Auxin and Cytokinin Combination in Nutrient Medium for Establishment of Optimal *in vitro* Multiple Plantlet in *Ficus carica* L. cv Siyah Orak

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ABSTRACT

Ficus carica Linnaeus is a flowering plant under the Moraceae family, usually propagated conventionally from cuttings due to the seeds being non-viable. However, this method is prone to diseases, and pests, time-consuming and space-intensive. Therefore, other methods are needed to overcome these issues. This study was conducted to induce callus and multiple shoots via plant tissue culture techniques enabling mass production of fig plants. Initially, leaf segments of *Ficus carica* L. cv Siyah Orak were cultured on different MS media strengths (¼, ½, ¾,1 MS) to induce callus. The highest callus means weight was observed on explant cultured in ¾ MS media (875±0.036). Callus was proliferated by subculturing explant into ¾ MS media supplemented with different concentrations of TDZ (0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L). MS media (3/4) supplemented with 2.0 mg/L TDZ (920±0.03) shows the best result for callus proliferation. Callus induction using transverse and longitudinal thin cell layers from nodal segments cultured on different MS media strengths (¼, ½, ¾,1 MS) shows ¼ MS as the optimum media for both tTCL (100±0) and lTCL (96.7±0.15). Friable callus (%) was observed the highest on ½ MS (63.33±0.55) and ¼ MS (76.67±0.50) media for both tTCL and lTCL, respectively. As for the number of leaves produced, both tTCL (0.83±0.0.28) and lTCL (1.00±0.33) explant showed the best results in ¼ MS media. Apical buds produced the highest mean for both the number of leaves and length of the shoot on 1MS media supplemented with 2.0 mg/L BAP (3.5±0.20, 13.73±0.66), respectively. For root formation (%) and number of roots, both show the best results in media supplemented with 2.5 mg/L IAA (10±0.31, 0.83±0.50). It can be concluded that the best shoot growth performance was observed from apical bud cultured on 1MS media supplemented with 2.0 mg/L BAP+ 2.5 mg/L IAA.

Key words: Apical buds *Ficus carica* L.cv Siyah Orak, leaf segment, nodal segment, thin cell layer

Article History
Accepted: 8 November 2023
First version online: 15 December 2023

Cite This Article:
Justin, M., James Antony, J.J., Embu, E.A. & Subramaniam, S. 2023. Optimization of different auxin and cytokinin combination in nutrient medium for establishment of optimal in vitro multiple plantlet in *Ficus carica* L. cv Siyah Orak. Malaysian Applied Biology, 52(5): 35-40. https://doi.org/10.55230/mabjournal.v52i5.cp19

INTRODUCTION

The Moraceae family includes the edible fig plant (*Ficus carica* L.), which is indigenous to the Middle East and South Asia and is valued in several civilizations all over the world. Both fresh and dried fig fruits are edible. In addition to being used as a food source, certain *Ficus* species are utilized as medicines in Ayurvedic and traditional Chinese medicine (Lee *et al*., 2022). Fig is said to have nutritional value to humans as it is cholesterol-free and an excellent source of minerals, vitamins, and carbohydrates. According to Mawa *et al*. (2013), it is also used for medicinal purposes and its consumption has effects on gastrointestinal, inflammatory, respiratory as well and cardiovascular disorders. Although the fig tree is indigenous to Central Asia, it has spread across the Mediterranean region, where it has adapted to a variety of soils and temperatures due to its drought and salinity tolerance.

As a result, figs are produced in many places of the world with moderate climates (Pereira *et al*., 2015). The fruit of the fig tree forms from the axillary bud. The fig fruit is

commonly referred to as false fruit or multiple fruits as it is a modified inflorescence. It has an ostiole which acts as a small opening that allows genera of fig wasps from the family of Agonidae to enter and pollinate (Weiblen, 2002). Due to the non-viability of the seeds, fig trees are often propagated using traditional techniques such as cuttings, air layering, and grafting. However, these techniques are prone to bacterial infections, pests, viruses, fungi, and illnesses (Chan Hong *et al*., 2020). It takes a lot of time and has a low success rate (Chan Hong *et al*., 2020).

Micropropagation is an alternative method to propagating fig trees. This method uses an explant for the proliferation and multiplication of *Ficus carica* L. for mass propagation at a high survival rate. Due to the totipotency property, all explants should have the same characteristics as the mother plant including their nutritional and medicinal value (Avato *et al*., 2005). Tissue culture techniques will result in disease and virus-free cultures. It also requires a relatively shorter duration, allows mass propagation, and is grown in a controlled environment (Sriskanda *et al*., 2021). Hence, this study was carried out to determine the optimal Murashige and Skoog (MS) media strength, hormone combination, and explant type to produce multiple plantlets of *Ficus carica* L. cv Siyah Orak.

MATERIALS AND METHODS

Plant material

Ficus carica L. cv Siyah Orak was obtained from HighTech Nursery, Miri, Malaysia. The plants were planted in pots and left outside of the Tissue Culture and Cryopreservation Laboratory, Universiti Putra Malaysia, Bintulu Campus, Sarawak. Explants included healthy leaves, nodal segments, and apical buds. The explants were excised from the mother plant and washed properly using tap water, followed by washing using Dettol and Tween 20 and then thoroughly rinsed with tap water thereafter. All explants were cultured in various media compositions (¼, ½, ¾, 1 MS) supplemented with different concentrations and types of hormones. and incubated at 25±2 °C under fluorescent lamps at 16 h photoperiods for 3 weeks. All experiments consisted of 6 replicates per treatment, with 5 samples of explant each.

Evaluation of the media strength types for the highest callus induction

Leaf segment and thin cell layer (TCL) explants were cultured on different MS media strengths (¼, ½, ¾, 1 MS). Each media was supplemented with 20 g/L sucrose, 2.75 mg/L gelrite, 1.0 mg/L activated charcoal, and 1.0 mg/L BAP with 0.2 mg/L IAA. Results on callus induction percentage and callus proliferation were recorded for leaf segments. Callus induction, friable callus percentage, and the number of leaves were evaluated for both transverse (tTCL) and longitudinal (lTCL) cell layers, respectively.

Evaluation of different hormone concentrations for callus proliferation of leaf segment, shoot, and rooting system for apical buds

Callus proliferated from the previous experiment of leaf segments were subcultured to ¾ MS media since ¾ MS shows the best results for callus induction. The media were supplemented with different concentrations of the hormone Thidiazuron, TDZ (0, 0.5, 1.0, 1.5, 2.0, 2.5, & 3.0 mg/L). Results on the weight of callus proliferated were taken. Apical buds were cultured on 1 MS media supplemented with different concentrations of hormone 6-Benzylaminopurine, BAP(0, 1.0, 1.5, 2.0, 2.5, & 3.0 mg/L). Results on the number of leaves and shoot length were taken. Media supplemented with 2.0 mg/L BAP shows the best results. Apical buds from the previous experiment were then sub-cultured into 1MS media supplemented with 2.0 mg/L BAP with different concentrations of Indole-3-acetic acid, IAA (0, 1.0, 1.5, 2.0, 2.5, & 3.0 mg/L). The results on the percentage of root formation and number of roots were taken.

Statistical analysis

All data were analyzed by one-way variance analysis (ANOVA). Significant differences ($p<0.05$) between treatment methods were tested using the Duncan Multiple Range Test at 5% probability using Statistical Analysis System (SAS) version 9.4.

RESULTS AND DISCUSSION

Effect of MS media strength on callus induction of leaf segment

A significant difference in the callus induction percentage of leaf segments was observed among different MS media strengths after four weeks of incubation (Table 1). The highest callus mean weight was noted on the leaf segment cultured in ¾ MS (875±0.03 mg) media supplemented with 1.0 mg/L BAP with 0.2 mg/L IAA. This finding is consistent with that of Wani *et al*. (2014) who reported the growth of callus was significantly higher at the lower medium strength (¼ MS) compared with ½ MS on the optimization of MS media strength for callus induction of *Costus pictus*.

However, in this study, the callus induction percentage of the leaf segment did not positively correlate with the increasing media strength. The use of MS media strength at 1 MS (660±0.037) significantly reduced the callus induction of the leaf segment compared with ¾ MS (875±0.03 mg). This could be due to a higher amount of nutrients can cause the explant to eventually die due to excessive nutrient uptake from the media, while an insufficient amount of nutrient will inhibit the growth. However, this finding is not consistent with Groll *et al*. (2002) in their experiment regarding the effect of medium concentration on callus induction of Cassava (*Manihot esculenta* Crantz) tested in different MS media strengths (½ & 1 MS).

This could be due to different types of plants having different nutrient requirements, where some may need higher concentrations of nutrients to grow. Based on his findings, cassava showed better callus results in 1 MS media compared to other media strength tested. Therefore, our findings suggest that ¾ MS media is the most optimum media strength that can be used to improve the callus formation of fig.

Table 1. Callus weight obtained about different MS media strength

Media strength (MS)	Callus weight (mg)
¼	697±0.047^{bc}
½	762±0.036^{b}
¾	875±0.036^{a}
1	660±0.037^{c}

Effect of different hormone concentrations on callus proliferation of fig leaf segments

Based on Table 2, there were significant differences in the mean of callus weights for leaf segments cultured on media supplemented with different concentrations of hormone. From our findings, ¾ MS media supplemented with 2.0 mg/L TDZ produced the highest callus mean weight (920±0.04 mg) for shoot proliferation, suggesting that 2.0 mg/L is the optimum TDZ concentration that can be used to produce callus from leaf segments of *Ficus carica* L. cv. Siyah Orak.

However, our finding contradicts that of Sa'adan and Zainuddin (2020) who reported that when they tested the callus induction from the leaf explant of *Ficus deltoidea* varkunstleri, the callus formation of the leaf explant was bigger and healthier as the concentration of BAP used increased (0.5, 1.0,1.5, 2.0, 2.5, & 3.0 mg/L).

Nevertheless, this finding is consistent with that of Liu *et al*. (2018), who reported that when they tested the effects of different hormone concentrations (0.5, 1.0, 2.0 mg/L BAP) on callus induction of *Rosa hybrida* L., it was noted that best callus was observed on 1.0 mg/L and not at higher concentrations. According to Long *et al*. (2022), the ability of plant regeneration is affected by multiple factors such as the use of plant growth regulators, composition of the basic medium, and explant type. Hence, the difference in the result obtained might be due to the difference in the type of hormone used.

Table 2. Callus weight obtained about different concentrations of TDZ

TDZ concentration (mg/L)	0	0.5	1.0	1.5	2.0	2.5	3.0
Callus weight (mg)	320±0.035^{e}	412±0.037^{d}	626±0.034^{c}	728±0.012^{b}	920±0.03^{a}	798±0.025^{b}	766±0.045

Effect of MS Media Strength for tTCL and lTCL Nodal Segment

Optimization of the different media strengths revealed significant differences in the callus induction (%), friable callus induction (%), and number of leaves for both tTCL and lTCL techniques. Results revealed that ¼ MS strength had the highest callus induction percentage for tTCL (100%±0) and lTCL (96.7%±0.15) segments. Significantly friable callus was obtained for tTCL segments cultured on ½ MS media (63.33%±0.55) and lTCL segments cultured on ¼ MS media (76.67%±0.15). Cultures on ¼ MS media produced the greatest number of leaves for both tTCL (0.83±0.28) and lTCL (1.00±0.33) (Table 3).

This study revealed that TCL shows optimum results when using lower MS media strength due to the explant having greater surface area contact resulting in efficient transport of medium compared to another type of explant (Da Silva & Dobránszki, 2019). Hence, using higher MS media strength could cause the death of explants due to stress especially for thin cell layer explants because of its large surface area. However, our findings contradict that of Tripathi *et al*. (2018) who reported that among all the media strength tested (1, ¾, & ½ MS), full-strength media gave the highest shoot initiation response for *Withania coagulans* Dunal.

In this study, 1/4 MS is the optimum media for the callus induction (%), friable callus induction (%), and number of leaves for both tTCL and lTCL techniques. However, lTCL explants show the optimum result for friable callus induction (%) in ½ MS.

Table 3. Callus induction, friable callus induction percentage, and number of leaves for tTCL and lTCL segments

Media strength (MS)	Callus induction(%)		Friable callus(%)		Number of leaves	
	tTCL	lTCL	tTCL	lTCL	tTCL	lTCL
¼	100±0^{a}	96.7±0.15^{a}	26±0.26^{b}	76.67±0.50^{a}	0.83±0.0.28^{a}	1.00±0.33^{a}
½	86±0.30^{ab}	76±0.43^{b}	63.33±0.55^{a}	46±0.55^{bc}	0.33±0.19^{b}	0.50±0.20^{b}
¾	70±0.36^{bc}	60±0.36^{b}	53±0.28^{a}	40±0.28^{bc}	0.16±0.15^{c}	0.37±0.15^{c}
1	56±0.33^{c}	50±0.43^{c}	0±0^{c}	0±0^{d}	0±0^{d}	0±0^{d}

In the table, the mean comparison is by column

Effect of different BAP concentrations on shoot formation of fig apical buds

Apical bud cultured on 1MS media supplemented with 2.0 mg/L BAP produced a higher number of leaves (3.50±0.20) and shoot length (13.73 mm±0.65) than other treatments (Table 4, Figure 1). From our findings, the shoot formation of fig apical buds does not correlate with the different concentrations of BAP hormone (0, 0.5, 1.0, 1.5, 2.0, 2.5, & 3.0 mg/L) suggesting that 2.0mg/L is the optimum media for shoot formation of fig Siyah Orak.

This finding is in agreement with Parab *et al*. (2021) who reported that the micropropagation of *Ficus carica* L. cv Black Jack supplemented with the different concentrations of BAP (0.2, 0.4, 0.6, 0.8, & 1.0 mg/L) does not correlates with the amount of shoot formed. Both experiments showed BAP was successful in inducing multiple shoots on apical bud explant. However, it is noted that the Parab *et al*. (2021) experiment used Woody Plant Medium (WPM). This finding is also consistent with that of Borthakur *et al*. (2011), who reported that the *in vitro* regeneration from apical buds of *Albizzia odoratissima* (L.f.) Benth when tested on different concentrations of BAP (0.25, 0.50, 0.75, & 1.0 mg/L) shows best result in media supplemented with 0.75 mg/L which also does not correlate with the amount of shoot formed. In this study, media supplemented with 2.0 mg/L BAP was the most optimum for shoot formation and shoot length.

Table 4. Number of leaves and shoot length of *in vitro* apical bud

BAP concentration (mg/L)	0	1.0	1.5	2.0	2.5	3.0
Number of leaves	1.16±0.23^{c}	2.16±0.21^{bc}	2.33±0.30^{b}	3.5±0.20^{a}	2.67±0.30^{b}	2.33±0.30^{bc}
Shoot length (mm)	9.35±0.37^{d}	10.50±0.35^{cd}	11.37±0.57^{cb}	13.73±0.66^{a}	12.50±0.51^{ab}	11.87±0.88^{bc}

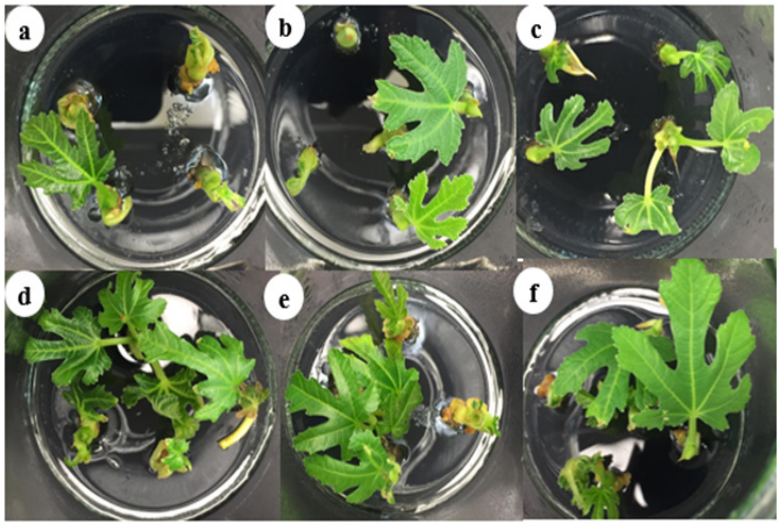

Fig. 1. Shows apical buds cultured on different concentrations of hormone BAP (mg/L). (a). Apical buds cultured on 0 mg/L, (b). Apical buds cultured on 1.0 mg/L, (c). Apical buds cultured on 1.5 mg/L, (d). Apical buds cultured on 2.0 mg/L, (e). Apical buds cultured on 2.5 mg/L, (f) Apical buds cultured on 3.0 mg/L after 3 weeks of incubation.

Effect of different IAA concentrations on percentage of root formation and number of roots produced from apical buds.

Different concentrations of IAA in 1MS media supplemented with 2.0 mg/L BAP induced different percentages of root formation (Table 5). Apical buds cultured on media supplemented with 2.0 mg/L BAP+2.5 mg/L IAA had the highest root formation (10%±0.31) and root number (0.83±0.50). Noticeable root formations were absent on all the other treatments tested (0.5, 1.0, 1.5, 2.0, & 3.0 mg/L IAA) except for 2.0 mg/L and 2.5 mg/L of IAA. However, it was observed that explants had shown signs of browning, and this indicates the presence of phenolic compounds that inhibit explants' growth (Dhage *et al*., 2015).

According to Soliman *et al*. (2010), lethal browning results from the oxidation of phenolic compounds which are released from the cut ends of explants or stress resulting in browning and hindering the explant establishment. This phenolic compound also promotes the formation of quinone which is toxic and highly reactive to plant tissue as reported by Titov *et al*. (2006). Titov *et al*. (2006) also reported that when testing on the control of phenolic compound secretion of *Musa* spp. Cv. Kanthali floral bud, explant cultured on media without any antioxidant treatment appeared to oxidize rapidly. Our findings contradict that of Sahraroo *et al*. (2019) who reported that the percentage and number of rooted explants increase as the concentration of indole-butyric-acid (IBA) reaches a certain point (1.5 mg/L) suggesting that 1.5 mg/L is the optimum IBA concentration for root number and percentage of fig cultivars 'Sabz' and 'Jaame-e-Kan'. Therefore, in this study, 1MS media supplemented with 2.5 mg/L IAA is regarded as the most optimal hormone concentration for both root formation percentage and number of roots produced.

Table 5. Root formation percentage and number of roots produced by apical bud

IAA concentration (mg/L)	0	1.0	1.5	2.0	2.5	3.0
Root formation (%)	0±0^{c}	0±0^{c}	0±0^{c}	3.33±0.15^{b}	10±0.31^{a}	0±0^{c}
Number of roots	0±0^{c}	0±0^{c}	0±0^{c}	0.33±0.30^{b}	0.83±0.50^{a}	0±0^{c}

CONCLUSION

The results concluded that the overall regenerations of *Ficus carica* L. showed the best shoot growth performance when using apical buds cultured on 1MS media supplemented with 2.0 mg/L BAP+ 2.5 mg/L IAA. The most optimum MS media strength for callus induction using leaf segment was observed on ¾ MS supplemented with 1.0 mg/L BAP and 0.2 mg/L IAA while for callus proliferation was on ¾ MS media supplemented with 2.0 mg/L TDZ and 0.2 mg/L IAA. In vitro, micropropagation of fig using a thin cell layer for both transverse and longitudinal technique were observed to produce the best callus in ¼ MS media when different MS media strength was tested. As for friable callus induction, ¾ MS media was observed to be the best among all other treatments tested using the transverse thin cell layer technique. However, the longitudinal thin cell layer technique was observed to have more friable callus in ½ MS media.

ACKNOWLEDGEMENTS

We would like to acknowledge the Institute of Ecosystem Science Borneo (IEB) and Universiti Putra Malaysia (UPM) for their support.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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