Journal of Tropical Plant Physiology 14(1) (2022):22-30
https://doi.org/10.56999/jtpp.2022.14.1.20

Callus Induction of Fig (*Ficus carica* cv. Violette de Soillès) via Thin Cell Layer Technique

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Received: 31 March 2022; Revised: 1 June 2022; Accepted: 16 June 2022; Published: 20 June 2022

ABSTRACT

Ficus carica L. is a fruit-bearing fig plant belonging to the Moraceae family. The fig fruit is known for its health benefits with high levels of fibre, vitamins, sugars and minerals. The leaves are also used for diabetes, cholesterol reduction and skin disorders. Previous reports have documented on the different *in vitro* callus induction methods of *F. carica* via plant tissue culture. Thin cell layer (TCL) has been previously established for the micropropagation of many plant species, utilising thin sections of explants in this method to induce callus. The current study aimed to evaluate the efficiency of the TCL technique in the callus induction of *F. carica* cv. Violette de Soillès. Stem explants with a diameter of 3.0 to 4.0 mm were excised into the thickness of approximately 0.5 to 0.8 mm and inoculated on MS media supplemented with BAP and NAA at different concentrations. The results indicated that MS media supplemented with 1.0 mg/L BAP and 0.5 mg/L NAA, and 2.0 mg/L BAP and 0.5 mg/L NAA induced the highest amount of semi-friable callus with 100% callus induction rate. The current study reported on the induction of callus from the Violette de Soillès cultivar via TCL, which in future, can be explored for shoot organogenesis, somatic embryogenesis and cell suspension culture.

Keywords: Callus induction, *Ficus carica*, micropropagation, thin cell layer, Violette de Soillès.

INTRODUCTION

Ficus carica L., commonly known as the edible fig, is a fruit-bearing plant belonging to the family of Moraceae. The species hosts a myriad of different cultivars distributed worldwide, devoid of climatic and geographic restrictions. The common fig is native to the South-Western parts of Asia, concentrated mainly in modern-day Turkey (Khatib and Vaya, 2010). Fig plants have a long lifespan of about 100 years and can grow vertically up to 15 m tall, although most stay between 3 to 9 m tall. In terms of botanical characteristics, the fig is not a fruit but an extension of the stem that expands into a receptacle with flowers that grow internally, known as the syconium (Lama et al., 2019). Fig fruits can be eaten fresh or dried and are traditionally applied in relieving soreness and inflammation (Patil and Patil, 2011). Lye or oral medication from the fig tree bark and the latex from the plant is able to treat skin warts (Veberic and Mikulic-Petkovsek, 2016). The leaves of the plant are also known for their anti-diabetic and cholesterol lowering effects (Mopuri and Islam, 2016) whereas certain extracts of figs can be used to prevent or even treat cancer (Lianju et al., 2003). On the other hand, the fig latex was found to inhibit the deregulation of Human Papilloma Virus onco-protein that is used as a diagnostic marker protein and has also proven to potentially reactivate tumour suppressor proteins (Ghanbari et al., 2019).

The cultivar of Violette de Soillès is part of the Bourjassote Noire variety that carries hints of acidity and sweetness, and is commonly grown in Provence-Alpes-Côte d'Azur (Allaway, 2017). The fruits are darker in colour compared to other fig cultivars, visually violet with dark veins that are dense and firm in texture whereas the flesh is of pinkish-red pulp colouration. The leaves have fine and serrated edges

(Allaway, 2017). The petioles of the Violette de Soillès figs are reddish at its base, differentiating them from other cultivars. Figs are commonly propagated via conventional methods such as cuttings. Unfortunately, these cuttings require large explants and they take up to 3 weeks for rooting to commence (Sarkhosh and Andersen, 2019). Due to the inefficiency of these conventional methods being applied for the propagation of plant stocks, micropropagation is often an alternative to overcome this matter.

In plant tissue culture, thin cell layer (TCL) technology has been studied on various plants for the propagation of plantlets and involves the excision of a small amount of transverse shoot explants. In a study conducted by Hanh et al. (2022) on the somatic embryogenesis of *Actinidia chinensis* Planch, TCL explants of the main vein (mv) and petiole (p) explants produced somatic embryos upon 8 weeks of inoculation. A total of 10.66 somatic embryos per explant were induced in ½ mv-transverse TCL explants, whereas ½ p-transverse TCL explants produced 8.66 somatic embryos per explant after 8 weeks of culture on MS medium incorporated with 0.02 mg/L NAA and 0.5 mg/L thidiazuron (TDZ). With reference to the work of Sabooni and Shekafandeh (2018), TCL was also applied for the propagation of two native blackberry plant genotypes of Iran and they discovered that this method resulted in high callus yields that are friable. A separate study by Croom (2016) proposed a thin cell layer technique to support the propagation of *Bacopa monnieri L.* (Scrophulariaceae), a valued medicinal plant. Transverse TCL explants of 30-day-old protocorm-like bodies (PLBs) of *Cymbidium* Sleeping Nymph produced an average of 5 PLBs at a response rate of 83% upon 30 days of inoculation when the explants were inoculated into Knudson C Orchid (KC) medium supplemented with 5% (v/v) coconut water (Vyas et al., 2010). Hence, these studies have indicated the potential of TCL as an alternative method in generating a large number of plantlets and plant mass required for various purposes (Efferth, 2019). TCL has been previously applied on *F. carica* for the cultivar of Sabz' and 'Torsh' and this technique was proven efficient for callus induction and indirect shoot regeneration for *F. carica* (Abdolinejad et al., 2020). Hence, this study aimed to evaluate the callus induction potential of *F. carica* cv. Violette de Soillès explants via TCL technique for *in vitro* propagation of this cultivar.

MATERIALS AND METHODS

Explant source

Segments of stem explants were obtained from *in vitro* cultures of *F. carica* cv. Violette de Soillès maintained in MS medium (Murashige and Skoog, 1962) supplemented with 1.0 mg/L of 6-benzylaminopurine (BAP), 20 g/L of sucrose and 8 g/L of plant agar (Duchefa Biochemie, USA).

Preparation of callus induction media

MS media (Murashige and Skoog, 1962) were prepared by supplementing with different concentrations and combinations of BAP (0.5, 1.0 and 2.0 mg/L) and 1-naphthaleneacetic acid (NAA) (0.5, 1.0 and 2.0 mg/L) with the MS media as the control. The pH was adjusted to 5.7 and the media were autoclaved at 121°C and 105 kPa for 15 min. The sterilised media were then poured into Petri dishes prior to explant inoculation.

Preparation and inoculation of explants

Eight-week-old *in vitro* stem explants of *F. carica* cv. Violette de Soillès with the diameter ranging from 3.0 to 4.0 mm were excised into the thickness of approximately 0.5 to 0.8 mm using a sterile scalpel blade. The thin explant slices were then inoculated onto MS media supplemented with different concentrations and combinations of BAP and NAA. Five explants were placed into a Petri plate with triplicates for each treatment, and the experiment was repeated twice.

Growing conditions

All *in vitro* cultures were maintained in a controlled environment in a 16 h photoperiod under white LEDs (light-emitting diodes) light (Philips TLD 36W/865–6500K, 3070 lm) (Philips, China) and at 25 ± 2°C and humidity of 50 ± 10% (Extech, USA) were provided in the tissue culture room.

Analysis of data

The explants in all treatments were observed on a weekly basis. After 8 weeks of culture, the size of induced callus was determined on a visual basis, whereas the percentage and type of callus induced were calculated using the following formula:

$$\text{Percentage of callus induction} = \frac{\text{Number of explants with induced callus}}{\text{Total number of explants}} \times 100\%$$

Qualitative observation for the size of callus was scored as no callus growth (0), minimal callus growth (+); slight callus growth (++); moderate callus growth (+++) and high callus growth (++++) with reference to Suwanseree et al. (2019).

RESULTS AND DISCUSSION

In this study, callus was induced on the explants after week four of culture and callus proliferation was observed on the eighth week of inoculation. The results indicated that MS medium with the supplementation of 0.5 mg/L BAP and 0.5 mg/L NAA, 0.5 mg/L BAP and 1.0 mg/L NAA, 1.0 mg/L BAP and 0.5 mg/L NAA, and 2.0 mg/L BAP and 0.5 mg/L NAA rendered maximum callusing (100%) with the formation of semi-friable callus (Table 1). It was evident that the treatments with the combination of 1.0 mg/L BAP with 0.5 mg/L NAA and 2.0 mg/L BAP with 0.5 mg/L NAA resulted in a higher amount of callus induction based on qualitative observations on the size of callus (Figure 1C). Treatment with a high amount of BAP (2.0 mg/L) and NAA (2.0 mg/L) resulted in the inhibition of callus formation with similar results obtained for the control treatment (Table 1). Combinations of 1.0 mg/L BAP with 1.0 mg/L NAA and 2.0 mg/L BAP with 1.0 mg/L NAA were observed to induce the formation of compact callus (Figure 1B) on explants, whereas other combinations of BAP with NAA induced the formation of semi-friable callus (Figure 1D). Callus growth was moderate in the combination treatment of 0.5 mg/L BAP with 0.5 mg/L NAA.

The employment of TCL explants is an efficacious alternative to conventional explants primarily due to the larger surface area of contact between the explant and the culture medium, as the explant cells are more receptive to acquire medium components in inducing organogenesis or embryogenesis (Van, 2003; Teixeira da Silva and Dobránszki, 2019). TCL is a technique previously applied in the *in vitro* propagation of different plant species and is occasionally preferred because it requires a small amount of plant materials to generate a high volume of biomass suitable for micropropagation purposes (Hidayat, 2016). Moreover, the minimal requirement of explant materials to initiate *in vitro* organogenesis enables the micropropagation of rare and endangered species for germplasm conservation in the event of propagule scarcity. This technique is also an important component of plant tissue culture whereby this method provides various solutions for mass cloning of plants and genetic transformation (Teixeira da Silva and Dobránszki, 2013). For example, in an agriculture-based study by Nhut et al. (2003) on four different types of cereals and grass reported that TCL technology laid a ground for an effective introduction of agronomically vital traits in plants by genetic transformation which gave rise to highly controlled and repeatable organogenesis and morphogenesis. However, this depends on the location of excision as it was observed that shoot transverse TCLs were more responsive than the other methods tested (Nhut et al., 2003).

Table 1. Relative size of callus, percentage of callus induction and types of callus induced under combination treatments of BAP and NAA after 8 weeks of culture

Treatments (mg/L)	Relative size of callus	Callus induction (%)	Type of callus
MSO (control)	0	0.0	No callus
0.5 BAP + 0.5 NAA	+++	100.0	Semi-friable callus
0.5 BAP + 1.0 NAA	++	100.0	Semi-friable callus
0.5 BAP + 2.0 NAA	++	83.3	Semi-friable callus
1.0 BAP + 0.5 NAA	++++	100.0	Semi-friable callus
1.0 BAP + 1.0 NAA	+	96.7	Compact callus
1.0 BAP + 2.0 NAA	++	73.3	Semi-friable callus
2.0 BAP + 0.5 NAA	++++	100.0	Semi-friable callus
2.0 BAP + 1.0 NAA	++	93.3	Compact callus
2.0 BAP + 2.0 NAA	0	0.0	No callus

Relative size of callus, 0: no callus growth, +: minimal callus growth; ++: slight callus growth; +++: moderate callus growth; and ++++: high callus growth

The excision of plant materials into thin cell layers are generally sectioned into the transverse or longitudinal explants wherein both differ in terms of their direction of excision and explant thickness (Vudala et al., 2019). Transverse TCL explants excised from nodal segments of *Eclipta alba* produced *in vitro* shoots at 32.6 shoot buds per explant when inoculated in MS medium supplemented with 13.2 µM BAP and 4.6 µM kinetin (Singh et al., 2012). In a study conducted by Singh et al. (2009) on *Spilanthes acmella* L., transverse TCL explants excised from nodal segments produced high shoot regeneration (97%) and maximum shoot induction at 31.5 shoots per explant when inoculated into MS medium supplemented with 5.0 mg/dm^3 BAP. Chattopadhyaya et al. (2010) reported on the shoot regeneration of *Sesamum indicum* L. using transverse TCL explants of internodal segments, where the incorporation of 2.0 mg/L BAP and 0.5 mg/L NAA into MS medium produced the highest percentage of shoot induction (29.8 ± 0.695%) and highest shoots per explant (15.0 ± 0.14 shoots). Meanwhile, explants excised at 1 mm produced the highest number of shoots per explants, at 15.3 shoots with decreasing trend as the explant thickness increased to 2.5 mm (0 shoots), indicating the role of TCL explant thickness in determining the efficacy of shoot induction in *in vitro* sesame cultures (Chattopadhyaya et al., 2010). Furthermore, the utilisation of longitudinal TCL explants of *Brasilidium forbesii* (Hook.) was explored by Gomes et al. (2015), where 1 mm TCLs excised from 6 month-old protocorms cultured into Woody Plant Medium (WPM) incorporated with 2.0 µM BAP resulted in 77% new protocorms and 22.7 PLBs per explant. In the *in vitro* study of *Paphiopedilum callosum* var. sublaeve, shoot tip-derived transverse TCL explants inoculated on Modified Vacin and Went (MVW) medium supplemented with 1.0 mg/L TDZ produced a maximum percentage of regenerated PLBs (46.67 ± 6.67%) and 40.00 ± 5.16 shoots per explant upon 8 weeks of inoculation (Wattanapan et al., 2018). Padilha et al. (2015) performed the histological analysis in explants of *Acrocomia aculeate* and reported that callus induction via TCL initiated after 2 days of culture, adjacent to the vascular bundles. The authors reported that the treatment of 12.5 µM 2-iP or 12.5 µM BAP were observed to induce callus with the occurrence of somatic embryos.

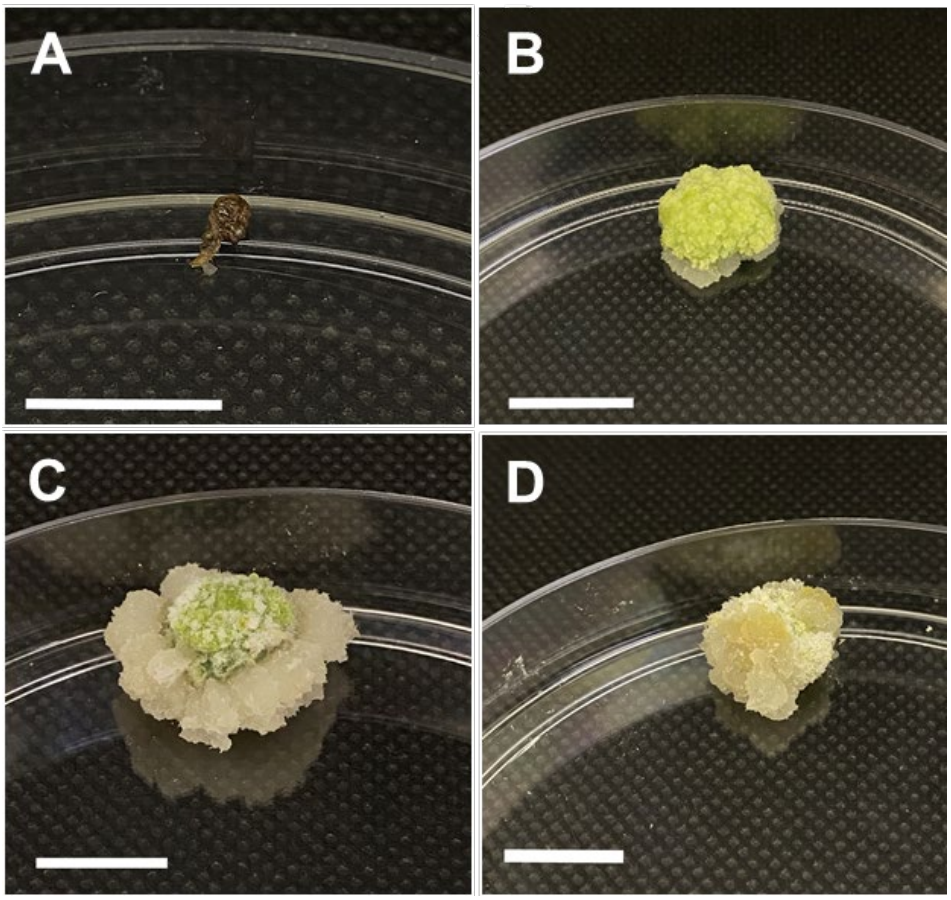

Figure 1. Callus induction from transverse TCL explants after 8 weeks of culture in different treatments of BAP and NAA. A) MSO showing no callus growth; B) MS media with 2.0 mg/L BAP and 1.0 mg/L NAA showing compact callus with slight growth C) MS media with 1.0 mg/L BAP and 0.5 mg/L NAA showing semi-friable callus with high growth; D) MS media with 0.5 mg/L BAP and 0.5 mg/L NAA showing semi-friable callus with moderate callus growth. Scale bars represent 1 cm.

Callus cultures are clusters of disorganised parenchymatous tissues caused by the strong proliferation of mitotic cell division in explant materials *in vitro* where the cells at the excised ends of an explant undergo mitosis, resulting in the formation of callus (Sridhar and Naidu, 2011). The efficacy of callus induction in *F. carica* explants have been explored by a few studies. Abdolinejad et al. (2020) reported on TCL technique for the explants of *F. carica* for the cultivars of 'Sabz' and 'Torsh' and the results revealed 50 ± 6.11% of callus regeneration in Murashige and Tucker (MT) medium supplemented with 9.08 µM TDZ plus 9.8 µM IBA. In this study, the morphogenic calli were further excised and cultured on MS medium supplemented with cytokinin (17.68 µM BAP with 4.54 µM TDZ) and auxin (1.07 µM NAA) combinations for shoot induction which resulted in shoot regeneration (6.9 shoots per explant). This gave rise to a basis for the potential of shoot regeneration via morphogenic callus for *in vitro* explants of *F. carica.* On the other hand, Dhage et al. (2013) observed the formation of callus for four fig cultivars namely Brown Turkey, Conadria, Deanna and Poona Fig from leaf explants of *in vitro* shoots. They reported the highest callus induction in MS medium supplemented with 2.0 mg/L TDZ and 4.0 mg/L 2-iso-pentenyl adenine (2iP) where the cultivar of Brown Turkey showed 85.8% callus induction as well as shoot induction in the same medium. In another study on the regeneration of *F. carica* from Iraq, the best medium for callus

induction was reported from leaf disc explants cultured in MS medium supplemented with 0.4 mg/L kinetin and 4.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) (Danial et al., 2014).

In this study, compact and semi-friable callus that were light green in colour (Figure 1B) were observed indicating the potential of these callus being used for shoot induction in the micropropagation of this cultivar. The exogenous addition and combination of cytokinin and auxin at equivocal proportions theoretically result in the formation of callus (Ikeuchi et al., 2013). In terms of the Violette de Soillès fig cultivar, a higher ratio of cytokinin to auxin was required to induce callus from TCL explants. A higher ratio of BAP to NAA correlated with an increase in the relative growth size of callus, which was in agreement with Kumlay and Ercisli (2015) on *Solanum tuberosum* L. and Das et al. (2018) on *Brucea mollis* Wall. ex Kurz. Moreover, MS medium supplemented with 0.5 mg/L BAP and 0.5 mg/L NAA successfully induced callus in chrysanthemum explants which proliferated in subsequent subcultures in the same culture medium (Ilahi et al., 2007). The formation of compact callus in the treatments of 1.0 mg/L BAP and 1.0 mg/L NAA, and 2.0 mg/L BAP and 1.0 mg/L NAA might be attributed to the role of cytokinins such as BAP in the transportation of nutrients. The osmotic potential inside cells is influenced by the cytokinin transport system whereas the turgor pressure is influenced by the presence of sucrose in the medium (Junairiah et al., 2017). This ultimately results in the formation of highly rigid cell walls that clump together to form compact callus. Additionally, callus is also the starting material for the initiation and establishment of cell suspension cultures where the accumulation of valuable secondary metabolites is often investigated. Given the wide range of secondary metabolites, antioxidants and anthocyanin found in *F. carica* as reported by Solomon et al. (2006), callus can be used for the establishment of cell suspension cultures for the production of valuable secondary metabolites in *F. carica.*

CONCLUSIONS

In this study, the combination treatments of 1.0 mg/L BAP with 0.5 mg/L NAA and 2.0 mg/L BAP with 0.5 mg/L NAA induced the highest amount of callus with the production of semi-friable callus that are light green in colour for *Ficus carica* cv. Violette de Soillès. The successful proliferation of these TCL-induced calluses in the Violette de Soillès cultivar posits as a significant contribution for further studies with regards to the production of novel metabolites via the exploitation of calluses through suspension culture. The proliferated calluses can be further investigated for various purposes, particularly indirect shoot organogenesis and somatic embryogenesis for large scale plant stock generation and to improve fig cell lines for the purpose of food security.

AUTHORS CONTRIBUTION

BLC conceived and designed the work. DS, XJC and BLC performed the analysis. BLC wrote the paper, and checked and approved the submission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

The authors would like to acknowledge the Economic Planning Unit, Prime Minister's Department for funding the project (Grant code: 304 /PBIOLOGI /6501099 /U120).

ACKNOWLEDGEMENTS

The authors thank Universiti Sains Malaysia for the research support and the Malaysian Superfruit Valley, Perlis for the supply of planting material.

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