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Establishment of *in vitro* Plant Regeneration Protocol for Fig (*Ficus carica* L.)

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ABSTRACT: Fig (*Ficus carica* L.) belongs to the family Moraceae is gaining momentum in the recent days due to its nutraceutical properties and health benefits. For farm establishment and small-scale home gardening, there is a rising demand for the plant propagules. Figs are commercially propagated by hardwood cuttings. In the present study, an attempt was made to standardize *in vitro* multiplication protocol for Fig. The explants were collected from the nodal part and treated with 0.5% carbendazim and 0.1 % streptomycin. Among the various sterilization treatments, treating the explants with 0.1 % $HgCl_2$ for 3 minutes recorded the highest aseptic culture establishment (72%). Nodal explants showed the highest bud initiation (65%) response in full strength MS medium supplemented with 3.00 mgL^{-1} of 6 – Benzyl Aminopurine with less time for shoot initiation (20 days). The highest number of multiple shoots (4) were observed in Murashige and Skoog medium fortified with 5.00 mgL^{-1}6 – Benzyl Aminopurine.

Keywords: Fig, nodal explants, sterilization, shoot initiation, shoot multiplication.

INTRODUCTION

Fig (*Ficus carica* L.) belongs to the family Moraceae, is a deep rooted tree and tolerant to salt and drought. The multiple fruit is botanically called Synconium and it is edible. The fruits are pear-shaped, variable in size, colour and contain lot of sugar. Vora *et al*. (2017) reported that fresh fruits are good source of carbohydrates (20.0 g/100g), proteins (1.02 g/100g), vitamin C (1.86 mg/100g), fibre (2.10 g/100g), calcium (104.2 mg/100g) and iron (0.725 mg/100g). Though fresh and processed figs are popular among the consumers, 90% of the fig produced is dried and consumed.

The major fig producing countries are Egypt, Turkey and Iran. In India, fig is widely cultivated in Maharashtra, Gujarat, Uttar Pradesh, Karnataka and Tamil Nadu (Sharma *et al*., 2022). Edible fig cultivar Brown Turkey is grown in Indian subcontinent. Its agronomical characteristics includes hardiness, drought tolerance and early commercial ripening have facilitated commercial crop expansion. The fruits are highly suitable for jams, canning and dried fruits have a good market value. Brown Turkey fruits also have exceptional quality characteristics such as fruit weight, size, firmness, flavour and sweetness. The nutritional value of this fruit also made it more appropriate for commercial production.

Figs are commercially propagated by cutting, grafting and layering in which only 20-30% survive (Kumar *et al*., 1998; Moniruzzaman *et al.,* 2015). In fig, mosaic virus, worms and mites are the major challenges faced (Bayoudh *et al*., 2015). The most significant need is the establishment of orchards with increased longevity using high quality planting material. Biotechnological tools can help to solve these issues and provides a faster way to mass propagate plants through tissue culture. *In vitro* propagation of *Ficus* species have been studied as an alternative method for large scale production of high-quality planting material (Rout *et al.,* 2006). *In vitro* regeneration in fig using various explants such as shoot tips, nodal explants, leaves and apical buds have been reported (Moniruzzaman *et al*., 2020). Micropropagation of figs provides a number of advantages over traditional vegetative propagation methods (Boliani *et al.,* 2019). It assures that the plants are true to type, superior in quality and disease-free planting materials are mass produced quickly irrespective of seasons (Sriskanda *et al.,* 2021). Hence, the present study was carried out to standardize the *in vitro* shoot proliferation protocol for Fig.

MATERIALS AND METHODS

The present study was carried out at Tissue Culture Laboratory, Department of Fruit Science, Horticultural

College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The explants were collected from two years old healthy and vigorously growing twigs of Brown Turkey variety from the Arid Zone Fruit Block, HC&RI, Coimbatore.

Preparation of explants and surface sterilization. Young and healthy twigs were collected from elite mother plants, the excess leaves were removed and the nodal explants of 2 cm were taken from the twigs. The excised explants were washed under running tap water thoroughly to remove the latex, adhering soil and dust particles. The nodal segments are then immersed in water containing surfactant (which increases the tissue penetration capability) polyoxyethylenesorbitan monolaurate (Tween 20) for 10 minutes and washed. The nodal explants were treated with fungicide 0.5% carbendazim for 30 minutes and bactericide 0.1 % streptomycin for 10 minutes along with two drops of polyoxyethylenesorbitan monolaurate which acts as surfactant. After this treatment, the explants were washed thoroughly in running tap water to remove the adhering chemicals completely. Under aseptic conditions in laminar air flow chamber, the explants were sterilized with ethanol for 10 seconds and then washed with sterile distilled water three times followed by sterilization with 0.1 % Mercuric Chloride for 1, 3, 5, 7 and 9 minutes and then washed thoroughly with sterile distilled water thrice to remove the chemicals completely.

Culture Medium. The most effective media for *in vitro* propagation of fig depends on the type of explant, cultivar and growth stage (initiation, multiplication and rooting). The Murashige & Skoog (1962) medium is widely used for the *in vitro* production of fig (Al-Shomali *et al.,* 2017). High salt concentrations in the MS medium enhances the tissue and cell growth. The explants were cultured on Murashige and Skoog (MS) basal medium supplemented with thiamine hydrochloride (0.1 mgL^{-1}), pyridoxine hydrochloride (0.5 mgL^{-1}), nicotinic acid (0.5 mgL^{-1}), glycine (2 mgL^{-1}), myo-inositol (100 mgL^{-1}), sucrose (30gL^{-1}) and antioxidants like citric acid (100 mgL^{-1}) and ascorbic acid (100 mgL^{-1}) were added to medium. 0.8% agar was added to the medium and pH was adjusted to 5.6 to 5.8.

Inoculation of explants. The sterilized nodal explants were inoculated into the culture tubes containing medium under aseptic condition in sterile laminar air flow chamber using scalpels and forceps. Fig shoots were inoculated in MS medium with 0.50, 1.00, 1.50, 2.00, 2.50and 3.00 mgL^{-1} BAP concentrations along with 0.2 mgL^{-1} NAA (Napthalene Acetic Acid) whereas MS basal medium without growth hormones served as the control. Each culture tube containing the nodal explants was tightly sealed with cotton plug and thin film. The inoculated culture tubes were kept in culture room consisting at 25±2°C temperature and 60-70% relative humidity. The culture tubes were maintained in a photoperiod of 16 hours light and 8 hours of darkness with 3000 lux light intensity using white fluorescent light. The cultures were checked periodically for bacterial, fungal contamination and phenolic exudates.

Shoot initiation. The initial procedure for *in vitro* plant establishment is initiation and the shoot initiation was observed after 20 days of inoculation. The highest shoot initiation was observed in MS media supplemented with 3.00 mgL^{-1} BAP. During the incubation phase, browning of explants was observed in few culture tubes and the culture tubes with contamination were removed subsequently.

Shoot proliferation. After four weeks of initiation, the explants were transferred to multiplication media. MS medium fortified with BAP at 4.00, 4.50, 5.00, 5.50 and 6.00 mgL^{-1} concentrations were used for multiplication of the explants. The sprouted explants with two or three leaves were transferred into the culture bottles containing multiplication medium. Shoots of 2.2 cm were observed after six weeks of inoculation. The shoots were later transformed into culture bottles containing different concentrations of media. Highest number of multiple shoots (4) were formed in MS medium containing 5.00 mgL^{-1} BAP.

Statistical Analysis. The experiments were conducted in Completely Randomized Design (CRD). Data was recorded at regular intervals and data was analysed using ANOVA at 5% level of significance.

Table 1: Effect of different duration of $HgCl_2$ treatment on establishment of aseptic culture in Fig.

Treatments	Aseptic Culture establishment (%)	Contamination (%)	Browning %
S_1- 0.1 % $HgCl_2$ for 1 minutes	31 (33.85)*	77 (61.37)	15 (22.79)
S_2 - 0.1 % $HgCl_2$ for 3 minutes	72 (58.08)	18 (25.11)	20 (26.57)
S_3 - 0.1 % $HgCl_2$ for 5minutes	52 (46.16)	13 (21.14)	55 (47.89)
S_4 - 0.1 % $HgCl_2$ for 7 minutes	11 (19.37)	48 (43.87)	61 (51.38)
S_5 - 0.1 % $HgCl_2$ for 9 minutes	3 (9.97)	60 (50.79)	72 (58.08)
SED	0.81	1.02	0.96
CD (0.05)	1.82	2.27	2.14
CV(%)	2.97	2.89	2.64

*The values given in parenthesis are arc sine transformed values

Table 2: Effect of different concentration of BAP and NAA on the shoot initiation of Fig.

Treatment	Shoot Initiation (%)	Time taken for shoot initiation (days)
M_1 - Basal MS	4 (11.54) *	65
M_2 - MS+ 0.50 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	12 (20.27)	59
M_3 - MS+ 1.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	21 (27.28)	51
M_4 - MS+ 1.50 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	33 (35.07)	47
M_5 - MS+ 2.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	43 (40.99)	34
M_6 - MS+ 2.50 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	58 (49.62)	28
M_7 - MS+ 3.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	65 (53.75)	20
SED	0.97	0.86
CD (0.05)	2.10	1.85
CV(%)	3.56	2.44

*The values given in parenthesis are arc sine transformed values

Table 3: Effect of different concentration of plant growth regulators on shoot multiplication of Fig.

Treatment	Days taken for multiple shoot induction	No. of multiple shoots/explant	Shoot length (cm)	No. of leaves/shoot
M_1 - MS+ 4.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	67	1	1.4	3
M_2 - MS+ 4.50 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	60	2	1.6	4
M_3 - MS+ 5.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	45	4	1.9	6
M_4 - MS+ 5.50 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	53	3	1.7	4
M_5 - MS+ 6.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA	64	2	2.0	2
SED	0.99	0.04	0.02	0.06
CD (0.05)	2.22	0.08	0.06	0.13
CV(%)	2.11	2.05	1.93	1.97

RESULTS AND DISCUSSION

Effect of duration of $HgCl_2$ treatment on aseptic culture establishment in Fig. One of the major challenges faced during aseptic culture establishment is the contamination (Wolella, 2017). The highest aseptic culture establishment (72.00 %) and the lowest contamination (18%) was recorded in the nodal explants treated with 0.1% $HgCl_2$ for 3 minutes (Table 1). The lowest aseptic culture establishment (3.00%) and the highest browning (72%) was observed in treatment S_5 - 0.10 % $HgCl_2$ for 9 minutes (Table 1). It was noticed that long exposure with the $HgCl_2$ decreased the survival rate. In the treatment S_1-0.1% $HgCl_2$ for 1 minute contamination percentage was high but the tissue death was low. Dhage *et al*. (2015) reported that treatment with 0.2 % $HgCl_2$ for 9 minutes recorded the best sterilization but the explant establishment percentage was low. Rattanpal *et al*. (2011) also reported that treatment of the explants with mercuric chloride (0.1%) for 4 min was the most effective surface sterilization procedure for maximum survival of explants with minimum tissue injury. The ideal concentration and duration might have lead to greater absorption of $HgCl_2$, which sufficiently decontaminates both systematic and environmental systems. Decrease in concentration of disinfectant and duration of treatment resulted in high percentage of contamination while increase in concentration lead to browning of explants.

Effect of different concentration of plant growth regulators on shoot initiation of Fig. Parab *et al.* (2021) also reported that BAP is a strong cytokinin inducing shoot initiation in many *Ficus* species. The highest shoot initiation (65%) and earlier initiation of the shoots (20 days) was recorded in MS + 3.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA. The explants cultured on Basal MS medium did not show any growth activity but the addition of BAP along with NAA resulted in direct shoot bud initiation. The lowest (4%) shoot initiation was recorded in the control basal MS without plant growth regulators and it also took the highest days for shoot initiation (65 days) (Table 2). The shoot initiation was due to the role of cytokinin BAP in breaking the apical dominance (Ali *et al.,* 2017).

Effect of different concentration of plant growth regulators on the shoot multiplication of Fig. In many fig cultivars, the role of BAP as a powerful cytokinin in shoot multiplication is recorded well. The highest number of shoots (4 shoots) and less time for shoot multiplication (45 days) was found when the shoots are supplemented with MS + 5.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA. Highest shoot length (1.9 cm) and higher number of leaves per shoot (6) was also found in this treatment. The lowest shoot proliferation (1) response was reported in the treatment M1-MS + BAP 4 mgL^{-1} along with 0.2 mgL^{-1} NAA. This treatment recorded the poorest shoot proliferation response with the highest days taken for shoot multiplication (67

days) and the lowest shoot length (1.4 cm). The results are similar with the findings of Prabhuling and Huchesh (2018) where MS medium supplemented with 1.00 mgL^{-1} BAP + 0.10 mgL^{-1} NAA resulted in highest number of shoots 3.50 (shoot/explants), shoot length (4.10 cm) and leaves/shoot (5). These results suggest that shoot proliferation in some species may be promoted by the presence of an auxin together with cytokinin. Cytokinin play an important role in stimulating cell division as well as cell elongation (Ling *et al.,* 2018). The use of high cytokinin levels was one of the most effective methods to promote the formation of meristematic clusters. Increased shoot growth might be caused by the optimal concentration of BAP and NAA, indicating a powerful synergistic effect of BAP-NAA interaction (Al-Malki *et al*., 2010).

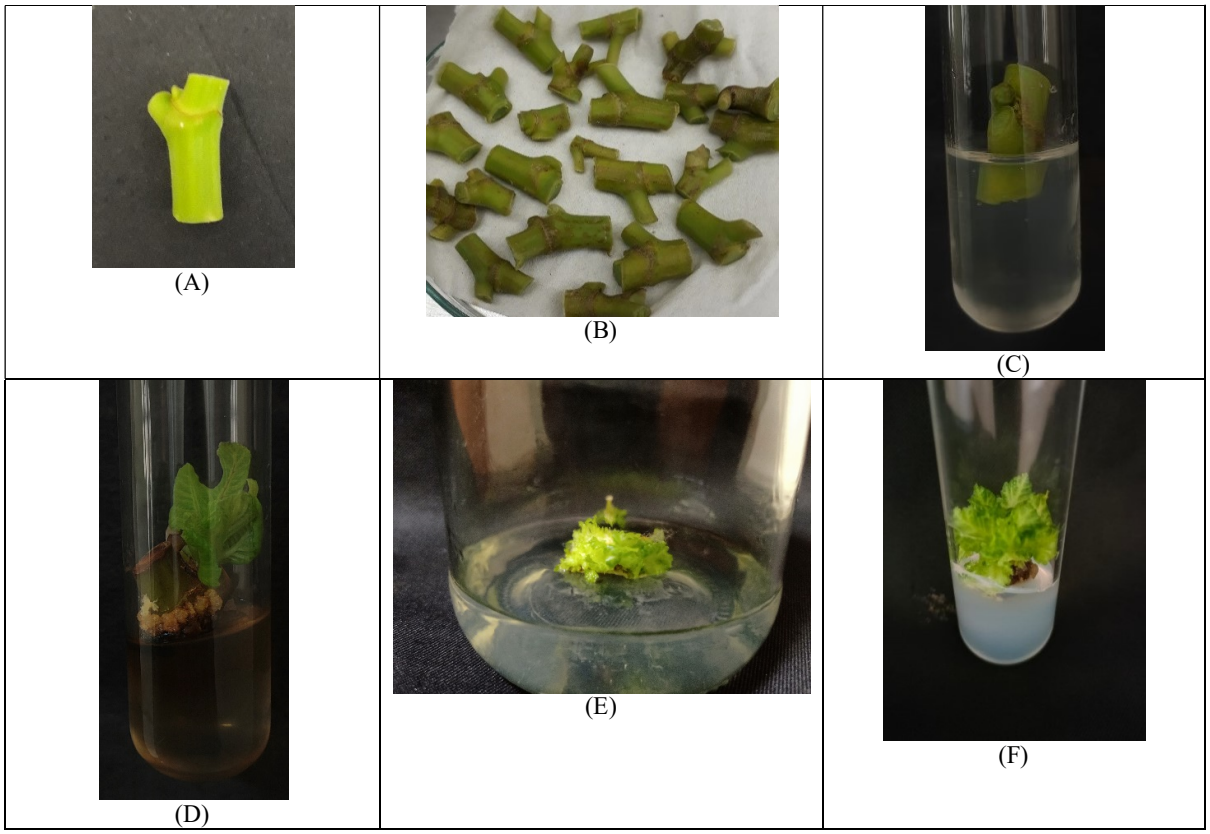

Fig. 1. A - Excised nodal explant; B - After surface sterilization with 0.1% $HgCl_2$; C - Inoculation in MS medium containing 3.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA; D - Shoot initiation in MS medium containing 3.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA; E - MS medium containing 5.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA; F - Multiple shoot formation in MS medium containing 5.00 mgL^{-1} BAP + 0.2 mgL^{-1} NAA

CONCLUSION

From the present study, it is concluded that in Fig variety Brown Turkey surface sterilization of the nodal explants with 0.1% $HgCl_2$ for 3 minutes recorded the highest aseptic culture establishment. MS medium supplemented with 3.00 mgL^{-1} BAP + 0.10 mgL^{-1} NAA produced highest shoot initiation. MS medium supplemented with 5.00 mgL^{-1} BAP + 0.10 mgL^{-1} NAA resulted in highest number of shoots, highest shoot length and leaves/shoot. Hence, this protocol can be utilized for mass multiplication of fig producing disease free and true to type planting materials for commercial production.

FUTURE SCOPE

On the basis of the present study, the protocol standardized have high potential for mass multiplication of plant propagules of fig. Future studies need to be carried out with different medium in order to study the *in vitro* shoot regeneration efficiency.

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Conflict of Interest. None.

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