Spectrum of Engineering Sciences
ISSN (e) 3007-3138 (p) 3007-312X Volume 4, Issue 5, 2026

INNOVATIVE MICROPROPAGATION AND CALLOGENESIS TECHNIQUES FOR SUSTAINABLE *FICUS CARICA* L. PRODUCTION SYSTEMS

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DOI: https://doi.org/10.5281/zenodo.20589820

Keywords
Ficus carica, Fig, Micropropagation, Callogenesis, MS medium, Acclimatization, Auxins and Cytokinins

Article History
Received: 11 March 2026
Accepted: 23 April 2026
Published: 08 May 2026

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Abstract
Ficus carica L. is a common edible plant, which is known by its economic and medicinal values with numerous bioactive substances having anti-diabetic, anti-bacterial, anti-cancer and anti-inflammatory activities. The contemporary study designed to establish an effective and effectual protocol for the mass production of F. carica through micropropagation and callogenesis. After surface-sterilization, explants were cultured on MS medium containing different plant growth regulators both auxins and cytokinin either alone or in combination to induce shoot proliferation, subsequent rooting and callus induction. Percentage of shoot induction observed was 95% in BAP 2 mg/L which was started within 12±0.43e days of inoculation and the maximum length of shoot 2.6±0.43^{a} cm with number of shoots 6±0.34^{a} whereas the maximum roots 5±0.44^{a} with highest length 2.5±0.54^{a} cm. In vitro plantlets of fig were effectively acclimatized in cocopeat having 65% survival rates in green house. The callus induction was optimized in 2, 4-D, BAP and NAA having equal amount of 0.5 mg/L. Percentage of callus formation was 95% which started within 40±0.20^{d} days after inoculation in media. Callus was white in color having hard and globular shape. The present study resulted in true-to-type stocks of Ficus carica appropriate for commercial usage.

INTRODUCTION

Ficus belongs to the family *Moraceae*, consists of more than eight hundred species comprising *Ficus carica* L. *Moraceae* is an important extant angiosperm plant family that is not only very rich in edible species but also characterized by milky latex in all its parenchymatous tissue, having unisexual flowers, anatropous ovules and aggregated drupes or achenes (Sharma et al. 2022; Vanmathi et al. 2022). Fig is a tree having deep fibrous roots and is drought and salt tolerant specie (Hussain et al. 2021). Common fig is cultivated for its edible pear-shaped fruits which are available in different colors and sizes as well as contains lots of sugar (Chan et al. 2021). Fig trees can be cultivated on almost all types of soil however, sandy loam and clay having pH 6.0-8.0 is considered to be the most favorite for fig cultivation (Faramayuda et al. 2022; Khan et al. 2018).

Vora et al. (2017) described that, the fresh fruits contain significant amounts of proteins (1.02 g/100g), carbohydrates (20.0 g/100g), ascorbic acid (1.86 mg/100g), various fibers (2.10 g/100g) and numerous minerals like iron (0.725 mg/100g), calcium (104.2 mg/100g). Turkey, Iran and Egypt are among the major fig producing countries (Shokoohi et al. 2022). In Pakistan, fig is generally cultivated in Pothwar region of Punjab, Gilgit Baltistan and Khyber Pakhtunkhwa (Soomro and Miano, 2023). Indian subcontinent is famous for edible fig cultivar 'Brown Turkey'. Its agronomical features include unique flavor, sweetness, hardness and drought tolerance (Erfa et al. 2022; Daniel et al. 2018). Fig fruit is commonly used for jams and canning; whereas it's dehydrated fruits contain extensive market worth (Naseer et al. 2020).

Ficus species which has significant commercial importance is *Ficus carica* L., it comprises of different varieties having important genetic diversity (Salhi et al. 2006). Commercial propagation of fig is generally through cutting, layering and grafting with only 20-30% survival rate (Sahraroo et al. 2019; Moniruzzaman et al. 2020). Mossaic viruses, mites or worms remain key factors that are responsible not only for decreased survival rate during propagation but also for poor quality of fig produced (Bayoudh et al. 2017). Therefore, it is the need of the time to establish propagation protocols for fig production with increased longevity (Sriskanda et al. 2021). This can be done by using efficient tools of biotechnology that are used to control such problems and offer a rapid way of mass production by tissue culture (Ling et al. 2022). *In vitro* micro-propagation of fig is considered an alternative technique for commercial propagation of high-quality fig trees (Tahiri et al. 2023). Micropropagation of fig has multiple benefits on traditional somatic propagation techniques (Boliani et al. 2019). For *in vitro* regeneration of fig, shoot-tips, nodal portion and leaves segments were studies (Moniruzzaman et al. 2021).

Callus culture offers a variety of advantages, including as a unique method for conducting various morphogenetic and physiological research, production of secondary metabolites, and production of beneficial somaclonal variants. Nowadays, there is significant potential for rapid replication of somatic embryogenesis using callus and suspension culture (Hesami et al. 2018). Extant literature shows that micropropagation of fig guarantees the true to type, disease-free or quality superior plantlets were produced. Apart from these salient features of micro propagation, plants are produced on commercial scale and in shorter time period irrespective of their season (Sriskanda et al. 2021).

Thus, the study is carried out to introduce a disease resistant, fast, low in cost, effective, and a simple method for the tissue culturing of *Ficus carica* through *in vitro* micropropagation and callogenesis protocols.

Materials and methods

1.1. Explant preparation and surface sterilization

The collection of explants from vigorously growing twigs of *Ficus carica* L. planted in Jinnah Garden, Lahore-Pakistan. From the top of the plant, the soft apical meristems and nodal parts were removed. After removing the explants' leaves and separating the apical meristem with a pair of fine scissors, shoot tips were cleaned with running tap water for few minutes in order to eliminate dirt particles without harming developing tissues. Liquid soap were used to surface sterilized the shoot tips then washed 5-6 times with distilled water and dipped into 25% sodium hypochlorite solution (bleach solution) for 30 minutes to avoid any contamination. After 30 minutes tips were washed with autoclave water (Ilyas et al. 2019).

1.2. Culture's medium Preparation

Meristematic and nodal portion were inoculated in MS (Murashige and Skoog, 1962) medium containing 0.01gL^{-1} thiamine hydrochloride, 0.05 gL^{-1} pyridoxine hydrochloride, 0.05 gL^{-1} nicotinic acid, 0.2 gL^{-1} glycine, 0.1 gL^{-1} myo-inositol or 30gL^{-}

[1] sucrose. 0.8% agar was added for solidification purpose and adjusts the pH to 5.5 to 5.7. Pre-sterilized culture tubes were filled with a volume of this medium, and the tubes were then covered through polythene bags with rubber bands. The medium was autoclaved for 20 minutes on 121°C and 151 psi for sterilization (Ilyas et al. 2019).

1.3. Explant inoculation

The explants were scalpel-cut to a particular size after surface sterilization. Take the apical meristem that has been inoculated into the MS media-filled test tubes under the sterile conditions of laminar air flow cabinets. Inoculated test tubes were labelled with the date of inoculation and the name of the variety of explant then incubated in a culture room containing 60-70% relative humidity or 22±2°C temperature. 16 hours light period or 8 hours dark period was maintained through 2000-3000 lux intensity with fluorescent light. Examined the cultures periodically and removed phenolic exudates or microbial contamination.

1.4. Acclimatization of plantlets

Plantlets with established roots were washed and placed in pots with varying ratios of coco-peat, vermiculite, perlite, farmland and soil and were kept at a high humidity level in green house. The plantlet's survival rate was checked on regular basis.

1.5. Statistical analysis

A total of three replicates of a randomized design were used. SPSS software, version 20.0 was applied to conduct the ANOVA (analysis of variance). Duncan's Test applied to equate mean values at 0.05%. All the values are the sum of three parallel replicates; ± SD indicates standard deviation among the replicates.

Results and discussion

1.6. Shoot induction and multiplication of *F. carica* with different PGRs

MS basal medium with various concentrations of 6-Benzyl aminopurine (BAP) mg/L were used for shoot tips and nodal portions inoculation of *F. carica*. The results related to shoot induction time (days), frequency of survival rate (%) and length of shoots are given in Table 1. 95% result of shoot induction was observed at 2.0 mg/L in which shoot induction initiated within 12±0.43^{e} days of inoculation and the shoot gained a maximum length of 2.6±0.43^{a} cm with number of shoots 6±0.34^{a} and number of roots 5±0.44^{a} with root length 2.5±0.54^{a} cm as shown in Figure 1 (a-c). This study suggested that BAP is the most potent plant hormone responsible for multiple shoot induction as well as maximum multiplication frequency and shoot length were observed in 2.0 mg/L BAP. Ling et al. (2022) was reported the same result as 2.0 mg/L BAP produced 100% shoot germination. Vanmathi et al. (2022) and Sriskanda et al. (2021) produced similar results and concluded that BAP is one of the strongest cytokinin responsible for shoot induction in *Ficus* species.

F. carica were also inoculated with constant concentration of BAP with different concentration of IBA Table 2. The shoot induction time ranged from 20 to 30 days. 70% survival rate was observed when 1.5 mg/L BAP and 0.2 mg/L IBA was used in which shoot initiated within 20±0.69^{e} days with number of shoots 4±0.89^{a}, shoot length 2.9±0.51^{a} cm, and number of roots 3±0.76^{a} with root length 3.4±0.43^{a} cm as shown in Figure 1 (d-f). Pratiwi et al. (2021) reported similar results.

F. carica were cultured in MS basal medium supplemented by constant concentrations of BAP whereas concentration of Kin was gradually increased from 0-1.5. The time for shoot induction ranged from 27-38 days. The best result (50%) was shown in BAP + Kin mgL^{-1} (Table 3). The survival rate was checked in 0.1 mg L^{-1} BAP and 0.5 mg L^{-1} Kin in which shoot induction initiated within 27±0.81^{e} days after inoculation with number of shoots 4±0.62^{a}, shoot length 2.2±0.82^{a} cm and number of roots 2±0.98^{a} with root length 2.0±0.23^{a} cm as shown in Figure 1 (g-i). These results are supported by the study directed by Danial et al. (2014).

Use another combination of hormones like BAP+ IAA mg L^{-1} (Table 4). The best survival rate (50%) was shown in MS medium contained 0.1 mg L^{-1} and 0.05 mg L^{-1} IAA in which shoot initiated within 30 ± 0.12^{e} days after inoculation with number of shoots 3.0 ± 0.48^{a}, shoot length 2.5 ± 0.62^{a} cm and number of roots 2 ± 0.54^{a} with root length 2.0 ± 0.69^{ab} cm as shown in Figure 1 (j-l). Study conducted by Ling et al. (2022) supported these results.

1.7. Acclimatization of regenerated plantlets

In vitro micro-propagated plantlets of *F. carica* were transferred to green house in different types and ratio of potting mixture which were *In vitro* micro propagated plantlets were shifted to greenhouse into four different types of potting mixture which were Farmland-vermiculite, cocopeat, cocopeat-vermiculite-perlite, perlite-cocopeat and soil as shown in Figure 2 (a-c). After acclimatization, the plant survival rate was examined on regular basis. However, the current judgments concluded that the individual cocopeat incorporation showed more satisfactory results for hardening of *Ficus carica* which was 65% survival rate in green house (Table 5). Studies conducted by Sriskanda et al. (2021) produced similar results.

1.8. Callogenesis of *F. carica* with different PGRs

In this part of the study, the protocols for callus induction from explant of figs were used. Young leaves of *F. carica* were inoculated in MS culture medium having various concentrations of 2, 4-D and BAP (mg/L) to induce callogenesis. The callus induction frequency ranged 50-90%. The highest callus (90%) was induced on MS culture medium supplemented by 1.0 mgL^{-1} 2, 4-D and 0.5 mgL^{-1} BAP in which callus formation initiated within 55 ± 0.61^{c} days after inoculation as shown in Figure 3 (a-f). The texture of callus was whitish green, friable as well as compact in nature as shown in Table 6. The medium enriched by 2, 4-D 1.0 mgL^{-1} and BAP 0.5 mgL^{-1}, however, proved to be the best and most standardized for callus development in *Morus alba* species (Dubey et al. 2020).

Young leaves of *F. carica* were also cultured in 2,4-D, BAP and NAA concentration for callus induction. 95% callus gained in MS medium having 0.5 mgL^{-1} of each of 2,4-D, BAP and NAA concentrations, in which callus formation initiated within 40 ± 0.20^{d} days after inoculation as shown in Figure 3 (j-i) which is supported by study directed by Danial et al. (2014). The obtained callus was hard and globular in shape and whitish in color as shown in Table 7.

Table 1: Effect of different concentrations of BAP (mgL^{-1}) on *in vitro* culture establishment of *F. carica*

Sr. #	Conc. of BAP (mg/L)	Shoot Induction Time (Days)	Survival Rate of Explant (%)	No. of Shoots	Shoot Length(cm)	No. of Roots	Root Length (cm)
1	0	20 ± 0.23^{a}	55	1 ± 0.21^{e}	0.6 ± 0.44^{bc}	1 ± 1.1^{d}	2 ± 0.23^{c}
2	0.5	17 ± 0.82^{b}	75	2 ± 0.98^{d}	1.0 ± 0.91^{b}	1 ± 0.39^{d}	2.2 ± 0.87^{bc}
3	1	15 ± 0.77^{c}	80	3 ± 0.65^{c}	2 ± 0.75^{a}	3 ± 0.81^{c}	2.0 ± 0.35^{c}
4	2	12 ± 0.43^{e}	95	6 ± 0.34^{a}	2.6 ± 0.43^{a}	5 ± 0.44^{a}	2.5 ± 0.54^{a}
5	3	13 ± 0.45^{d}	92	5 ± 0.32^{b}	2.0 ± 0.71^{a}	4 ± 0.37^{b}	2.3 ± 0.66^{b}

Spectrum of Engineering Sciences
ISSN (e) 3007-3138 (p) 3007-312X Volume 4, Issue 5, 2026

Table 2: Effect of different concentrations of BAP+IBA (mg L^{-1}) on *in vitro* culture establishment of *F. carica*

Sr. #	Conc. of Plant Hormones (mg/L)		Shoot Induction Time (Days)	Survival Rate of Explant (%)	No. of Shoots	Shoot Length (cm)	No. of Roots	Root Length (cm)
	BAP	IBA						
1	1.5	0	30 ± 0.91^{b}	50	3 ± 0.34^{b}	2.2 ± 0.39^{bc}	1 ± 0.43^{c}	2.2 ± 0.49^{b}
2	1.5	0.15	35 ± 0.88^{a}	40	2 ± 0.78^{c}	2.5 ± 0.76^{b}	2 ± 0.29^{b}	2.0 ± 0.88^{b}
3	1.5	0.2	20 ± 0.69^{e}	70	4 ± 0.89^{a}	2.9 ± 0.51^{a}	3 ± 0.76^{a}	3.4 ± 0.43^{a}
4	1.5	0.25	25 ± 0.63^{d}	40	1 ± 0.22^{d}	2.0 ± 0.48^{c}	1 ± 0.81^{c}	2.2 ± 0.89^{b}
5	1.5	0.5	29 ± 0.23^{c}	45	1 ± 0.43^{d}	2.1 ± 0. 33^{c}	3 ± 0.23^{a}	2.0 ± 0.17^{b}

Table 3: Effect of different concentrations of BAP+ Kin (mg L^{-1}) on *in vitro* culture establishment of *F. carica*

Sr. #	Conc. of Plant Hormones (mg/L)		Shoot Induction Time (Days)	Survival Rate of Explant (%)	No. of Shoots	Shoot Length(cm)	No. of Roots	Root Length (cm)
	BAP	Kin						
1	0.1	0	38 ± 0.56^{a}	40	1 ± 0.73^{c}	2.0 ± 0.83^{ab}	1 ± 0.54^{b}	1.9 ± 0.21^{ab}
2	0.1	0.5	27 ± 0.81^{e}	50	4 ± 0.62^{a}	2.2 ± 0.82^{a}	2 ± 0.98^{a}	2.0 ± 0.23^{a}
3	0.1	1.0	30 ± 0.78^{c}	25	1 ± 0.37^{c}	2.0 ± 0.71^{ab}	2 ± 0.78^{a}	1.6 ± 0.67^{b}
4	0.1	1.25	29 ± 0.44^{d}	35	2 ± 0.90^{b}	1.5 ± 0.49^{b}	1 ± 0.56^{b}	1.5 ± 0.32^{bc}
5	0.1	1.5	35 ± 0.68^{b}	40	1 ± 0.81^{c}	1.8 ± 0.82^{b}	1 ± 0.43^{b}	1.1 ± 0.78^{c}

Table 4: Effect of different concentrations of BAP+IAA (mg L^{-1}) on *in vitro* culture establishment of *F. carica*

Sr. #	Conc. of Plant Hormones (mg/L)		Shoot Induction Time (Days)	Survival Rate of Explant (%)	No. of Shoots	Shoot Length(cm)	No. of Roots	Root Length (cm)
	BAP	IAA						
1	0.1	0	45 ± 0.33^{a}	40	2 ± 0.82^{b}	2.0 ± 0.43^{c}	1 ± 0.12^{b}	1.9 ± 0.44^{b}
2	0.1	0.05	30 ± 0.12^{e}	50	3 ± 0.48^{a}	2.5 ± 0.62^{a}	2 ± 0.54^{a}	2.0 ± 0.69^{ab}
3	0.1	0.1	35 ± 0.99^{d}	40	2 ± 0.97^{b}	2.2 ± 0.91^{b}	1 ± 0.87^{b}	2.2 ± 0.45^{a}
4	0.1	0.2	38 ± 0.87^{c}	35	1 ± 0.46^{c}	2.1 ± 0.28^{bc}	1 ± 0.52^{b}	2.1 ± 0.89^{a}

5	0.1	0.3	40 ± 0.72^{b}	45	1 ± 0.42^{c}	1.5 ± 0.84^{d}	2 ± 0.65^{a}	1.5 ± 0.54^{c}

Table 5: Effect of different potting mixtures on acclimatization of *in vitro* regenerated plantlets of *F. carica*

Sr. #	**Different potting mixture**	**Frequency of plant survival (%)**	**Potting mixture (%)**	**Plant growth after acclimatization**
1	Farmland-Vermiculite	55	2:1	Good
2	Cocopeat	65	-	Excellent
3	Cocopeat-Vermiculite-Perlite	60	1:1:1	Good
4	Perlite-Cocopeat	62	1:1	Good
5	Soil	50	-	Fair

Table 6: Effect of different concentrations of BAP+2,4-D (mgL^{-1}) on callogenesis of *F. carica*

Sr. #	**Conc. of Plant Hormones (mg/L)**		**Callus Formation (Days)**	**Percentage of Callus Formation**	**Texture of callus**	**Color of callus**
	2, 4-D	**BAP**				
1	0	0.5	No Callus	-	-	-
2	0.5	0.5	59 ± 0.49^{b}	80	Friable and compact	Light Brown
3	1	0.5	55 ± 0.61^{c}	90	Friable and compact	Whitish Green
4	2	0.5	60 ± 0.77^{a}	60	Friable and compact	Brown
5	5	0.5	40 ± 0.56^{d}	50	Friable and compact	Brown

Table 7: Effect of different concentrations of BAP+NAA+2,4-D mgL^{-1} on callogenesis of *F. carica*

Sr. #	**Conc. of Plant Hormones (mg/L)**			**Time for Callus Formation (Days)**	**Percentage of Callus Formation**	**Texture**	**Color**
	BAP	**NAA**	**2, 4-D**				
1	0	0.5	0	No callus	-	-	-
2	0.5	0.5	0.5	40 ± 0.20^{d}	95	Hard and globular	White
3	1	0.5	1	60 ± 0.58^{a}	80	Hard	Off-white
4	2	0.5	2	55 ± 0.91^{b}	79	Hard	Off-white
5	3	0.5	3	50 ± 0.45^{b}	92	Hard	Off-white

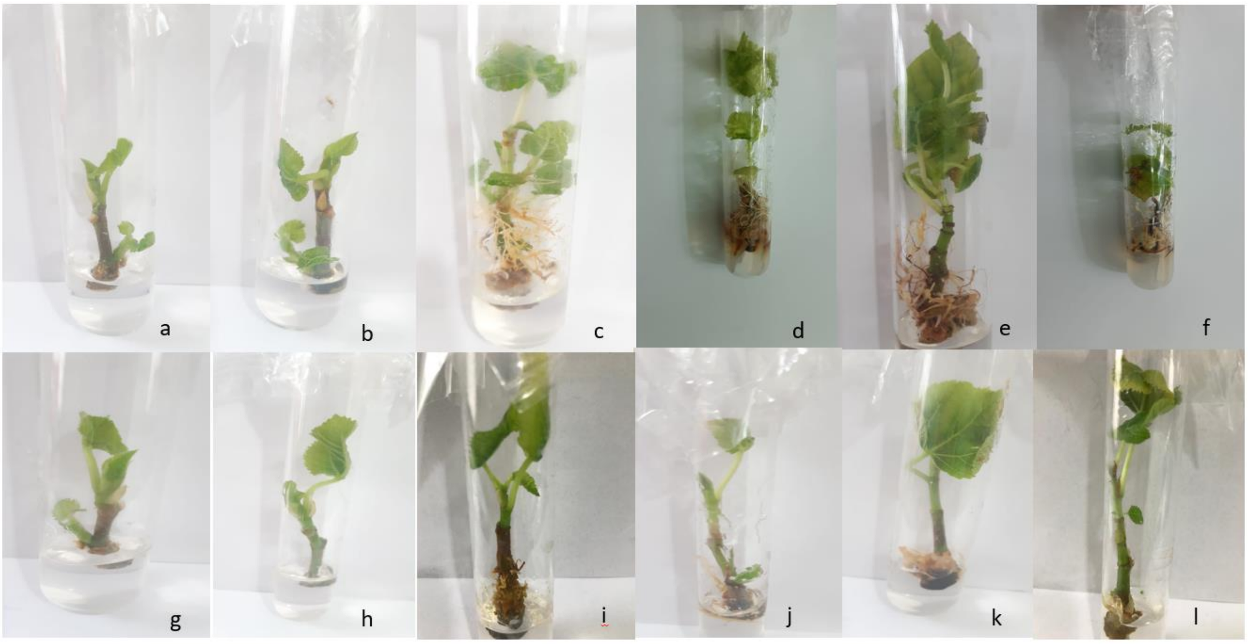

Figure 1 (a-c): Effect of BAP mgL^{-1} on shoots induction, shoot multiplication and root induction *F. carica.* Shoot induction started within 12 days (d-f): It showed effects of IBA and BAP on shoot induction, shoot multiplication or root induction of *F. carica* (g-i): It showed influence of BAP and Kin mgL^{-1} on induction of *F. carica* shoots (j-l): It showed effects of IAA and BAP mgL^{-1} on induction of *F. carica* shoots.

Figure 2 (a-c) Effect of cocopeat on *Ficus carica* plantlets (d-f) Effects of different concentrations of potting mixture in green house

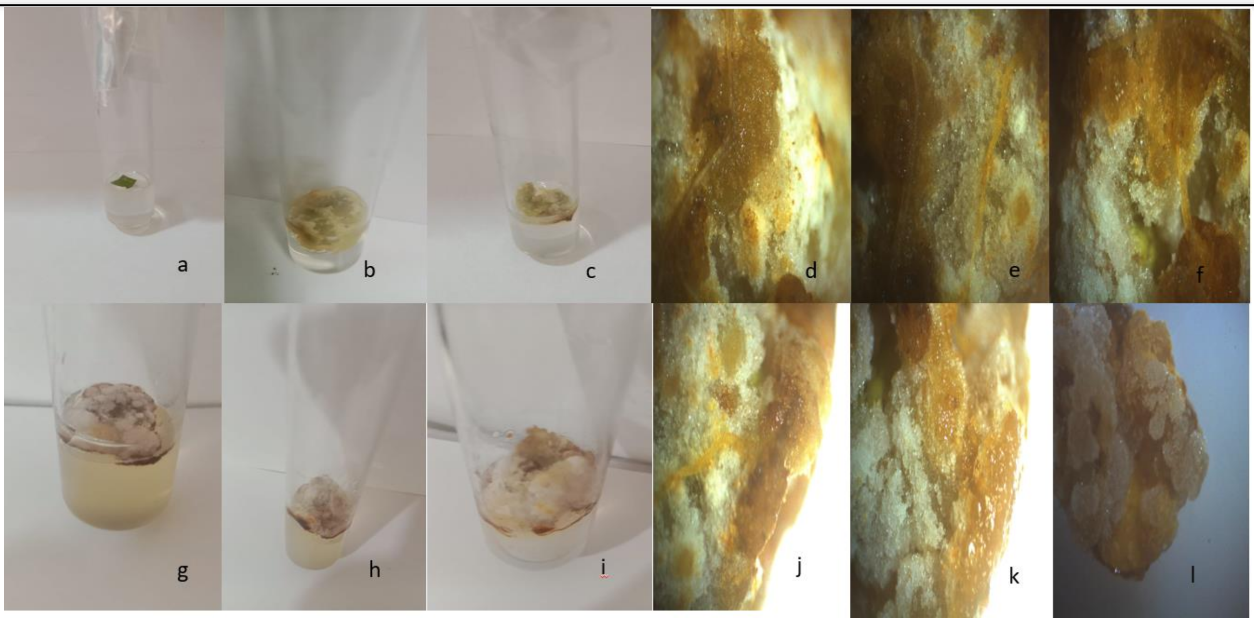

Figure 3 (a-f) Showed impact of BAP +2,4-D mgL^{-1} on callogenesis of *F. carica* and (d-f) showed 2X image of greenish callus which was compact and friable in texture (g-i) showed impact of BAP+NAA+2,4-D mgL^{-1} on callogenesis of *F. carica* and (j-l) showed 2X image of whitish callus which was hard and globular in texture.

Conclusion

This investigation highlights the multifaceted value of *Ficus carica*, encompassing its medicinal, nutritional and commercial values. The successful application of plant tissue culture techniques yielded a substantial number of disease-free fig plantlets in a relatively short period. This approach exhibits considerable potential for mass multiplication and true-to-type planting material production, thereby supporting the commercial cultivation of high-quality fig crops. Our study concluded that BAP is the most efficient plant hormone for shoot induction whereas IBA significantly induces root elongation. The study also suggests that no significant effects were obtained by inclusion of kinetin in the culture medium. It was also suggested that the combination of 2,4-D+BAP+NAA is an essential plant growth regulator for callus induction.

Acknowledgments

Authors would like to acknowledge Plant tissue culture laboratory Bagh-e-Jinnah for providing lab and facilities in conducting present work.

Author's Contribution

All authors have made their generous involvement in concept, strategy and design or data analysis for this article.

Conflict of Interest

No conflict of interest was declared by authors about the publication of this article.

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