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Organogenesis on apical buds in common fig (*Ficus carica*) var. Black Jack

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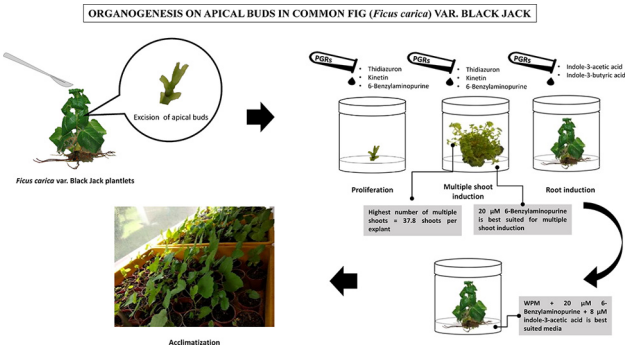
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Plant tissue culture involves the use of explants obtained from plants to induce organogenesis with the help of plant growth regulators (PGRs). Micropropagation techniques provide a faster and economical solution to the limitations associated with traditional methods of plant cultivation. The present study focuses on the multiple shoot induction and proliferation of *Ficus carica* var. Black Jack. Factors that influence the growth of *in vitro* multiple shoots on the apical buds, which include growth media and PGRs, were investigated in this study. Different concentrations of cytokinins like 6-benzylaminopurine (BAP), Thidiazuron (TDZ), and Kinetin (Kin) were used on woody plant medium (WPM) for the optimization of media for multiple shoot induction and proliferation.

Results: Apical buds of *Ficus carica* var. Black Jack growing in WPM supplemented with BAP produced the healthiest plantlets, with the highest number of multiple shoots. The most efficient medium composition which produced the highest number of multiple shoots (37.8) per growing explant was WPM supplemented with 20 µM BAP. Proliferated multiple shoots were efficiently rooted using WPM + 20 µM BAP + 8 µM indole-3-acetic acid (IAA). This optimized medium composition significantly enhanced the production of multiple, disease-free plantlets using single apical bud explants of *Ficus carica* var. Black Jack.

Conclusions: In the present study the observations indicate that WPM supplemented with 20 µM BAP is the best-suited medium for organogenesis and multiple shoot culture of *Ficus carica* var. Black Jack, and this technique can be potentially applied for commercialization of the plant.

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1. Introduction

The development of a successful micropropagation and plant regeneration system has become vital and it has been previously reported on different plants such as six endangered plant species from India [1], cultivars of barley [2], cucumber [3], and mulberry [4] using various explants and combinations of plant growth regulators (PGRs). Cytokinins such as 6-benzylaminopurine (BAP), Thidiazuron (TDZ), Kinetin (Kin), and many others are extensively utilised for *in vitro* plant regeneration [5]. Apical bud explants of fig variety 'Sultani' were cultured on growth media containing different PGRs, namely, Kin and BAP (0.5 and 1.0 mg/L). Although shoot proliferation was observed on all the media, a growth medium containing 0.5 m/L Kin produced the highest number of shoots [6]. Micropropagation studies were also carried out on other three endangered fig genotypes, 'Bargchenari', 'Dehdez' and 'Runu'. It was reported that 'Bargchenari' and 'Runu' produced the highest shoot proliferation in MS medium containing 0.5 mg/L benzyl adenine (BA) and 0.2 mg/L N6-(Δ2-isopentenyl)adenine (2ip), and 'Dehdez' produced the highest multiple shoot in a medium containing 6 mg/L Kin and 0.2 mg/L 6-α-naphthaleneacetic acid (NAA) [7]. The application of PGRs in the growth media activates the signalling pathways of the meristematic cells in the growing explants. The tendency of the meristematic cells to undergo directed organogenesis produces shoots and roots on the explants. Apical bud acts as an efficient explant for multiple shoot induction owing to the presence of meristematic cells [8,9]. Therefore apical buds have been used in many studies for *in vitro* regeneration for fig plants [10].

Fig (*Ficus carica* L.) is one of the important fruit trees, cultivated in South East Asia, especially in Malaysia. Figs belong to the Moraceae (Mulberry) family and there are over 900 known varieties of figs. Black Jack variety of fig is a variety of common fig, with darker colour fruits. Conventional methods like stem cuttings and air layering are applied for the production of figs in Malaysia. However, these methods are time-consuming and uneconomical. Besides, the occurrence of intricate problems relating to pests and diseases is common in the open-air cultivation of figs [11,12,13].

Micropropagation of three genotypes of figs from Jordan, 'Khdari', 'Mwazi' and 'Zrak', was done using three kinds of media, MS medium [14], WPM (Woody plant medium [15]), and OM (Olive medium [16]). Multiple shoots were observed on the apical bud explants of the three fig genotypes on all three media. However, OM produced a significantly high number of shoots [17]. In the case of the 'Panachee' cultivar of fig, plant regeneration was obtained using leaf explants, with a focus on the effects of photoperiodism and gravitropism [18]. *In vitro* propagation of fig cultivar, 'Salti kodari' was completed using half-strength MS medium (½MS) supplemented with different concentrations of BAP, Kin, and Zeatin. Successful plant regeneration was achieved, with the highest number of multiple shoot propagation observed on ½ MS medium supplemented with 0.4 mg/L BAP and 0.2 mg/L indole-3-butyric-acid (IBA) [19].

Although multiple shoot induction can single-handedly become the solution for a quicker plant regeneration system it is essential to optimise which plant hormone to be used. Generally, cytokinins produce a good response for shoot induction [5]. Many studies have used cytokinins such as BAP, TDZ, and Kin for shoot development in *Ficus carica* and in many other different fig cultivars such as 'Poona fig', 'Brown Turkey', 'Conadria' and 'Deanna' to establish shoots using apical buds as explants [20]. All plants produce a range of endogenous plant hormones, and varied responses are observed in different plants using different PGRs [21,22,23]. Therefore, optimization of growth media is an essential step, which helps to identify the most effective hormone for the plant species [10,24]. Subculturing the growing plantlets to a new medium to ensure the viability of the generating plantlets is another essential step and it reduces the risk of losing a good culture [25,26]. Despite the risk of losing a good culture, due to improper subculture techniques, *in vitro* plant regeneration systems have been proven to be successful in terms of higher yields [1,2,24].

Tissue culture on *Ficus carica* var. Black Jack was proposed to promote multiple shoot induction. The demand for Black Jack variety of figs is rising exponentially in the commercial markets in Malaysia. Thus, the production of multiple shoots for the macro-level commercialisation of the Black Jack variety of figs in Malaysia is deemed essential [27]. Along with the demand, the need to cultivate disease-free plants is also a priority [28]. Optimisation of medium composition plays a critical role in achieving a successful regeneration system. This will ensure high yields of disease-free plants of the Black Jack variety of figs. The main aim of the present study is to optimize medium composition for the production of multiple shoots on the apical buds of *Ficus carica* var. Black Jack using PGRs such as BAP, TDZ, and Kin and to obtain successful acclimatization of the rooted multiple shoots.

2. Materials and methods

2.1. Plant material and culture conditions

In vitro regenerated plants of *Ficus carica* var. Black Jack was chosen for this experiment. The most commonly used explants for the following experiments were the apical buds of these *in vitro* regenerated plants. Healthy apical buds were collected from a 2 year-old mature mother plant of *Ficus carica* var. Black Jack, which was maintained in the School of Biological Sciences, Universti Sains Malaysia (USM). The explants were initially washed and brushed with water, and then thoroughly rinsed under running tap water for 30 minutes, followed by a robust sterilisation protocol. The sterilisation protocol included sequentially washing the explants in 70% Ethanol, and 50% Clorox with 2 drops of tween 20 for 10 min each. This step was repeated twice, followed by washing the explants with sterile distilled water (8–10 changes) for each between 2 and 3 min. Apical buds were then cultured and grown on Woody Plant Medium (WPM) supplemented with 20 μM BAP and 8 μM IAA (indole-3-acetic acid). WPM was used as the basal medium to induce and proliferate multiple shoots on the apical buds of *Ficus carica* var. Black Jack (McCown Woody Plant Medium – DUCHEFA; 2.46 g/L) along with sucrose (15 g/L) for shoot induction from the apical bud explants. Gelrite was used as a gelling agent, 3 g/L. The pH of all media was adjusted between 5.7 and 5.8 prior to autoclaving at 1.05 kg/cm2, 121 °C for 20 min. After culturing the ~0.4 cm apical bud on the WPM, the i*n vitro* cultures were incubated at 25 ± 1 °C under white fluorescent light (Philips TLD (Low voltage (Tension Lag Dunn) thin lamps), 36 W, 60 $\mu mol.m^{-2}.s^{-1}$) for a daily 16-hour photoperiod.

2.2. Effects of cytokinins on shoot induction

Apical buds were cultured on WPM supplemented with different cytokinins (BAP, Kin, and TDZ). The concentrations used for each treatment were 5, 10, 15, 20, 25, and 30 μM. WPM without any growth regulators was used as the control medium treatment. Each culture jar consisted of five explants initially, and a total of thirty cultures were grown under each treatment. The data (number of shoots) were recorded after every four weeks of culturing. Growing plantlets were subcultured to a fresh culture medium after every four weeks. The subculturing process was carried out five times consecutively, and data for the number of shoots were recorded for each subculture.

2.3. Effects of 20 μM BAP on shoot proliferation

The explants used for shoot proliferation were the single shoots, previously acquired (Treatment T5) from the multiple shoot induction. Single shoot at the height of 2–3 cm was cultured on WPM supplemented with different concentrations of BAP (5, 10, 15, 20, 25, and 30 μM) as a growth regulator. The data (the number of shoots) were determined after every four weeks of culturing. The cultures were subcultured to a fresh medium every four weeks for five subcultures.

2.4. Root induction and acclimatization

The multiple shoots acquired from the prior experiment (M5 medium) were subjected to the production of roots. For the induction of roots, different concentrations of auxins such as IAA and IBA (2, 4, 6, 8, and 10 μM) were selected. WPM supplemented with 20 μM of BAP was used as the basal medium, for the optimisation of the rooting hormone for the multiple shoots of *Ficus carica* var. Black Jack. Three explants were cultured in one jar, and ten cultures were raised in each treatment. The number of roots was determined after 8 weeks of culturing. Plantlets with well–developed roots were used for the acclimatization process. The plantlets were removed from the jar and washed under tap water and transferred to plastic trays containing sterile soil (BioChar Soil Mix 1, Serbajadi). After 2 weeks of incubation under white fluorescent light (Philips TLD, 36 W, 60 $\mu mol.m^{-2}.s^{-1}$) for a daily 16 h photoperiod, the plastic trays were transferred outside the laboratory conditions and watered regularly for another 2 weeks. This method of acclimatization is used to produce disease-free micropropagated plants of *Ficus carica* var. Black Jack [29].

2.5. Statistical analysis

All experiments were repeated three times and each experiment contained five explants per jar and six jars per treatment. The data were reported as mean ± SE (standard error). Means were statistically analyzed by one-way analysis of variance (ANOVA) and treatment means were considered to be significantly different from controls after analysis with Tukey's multiple range test at $P < 0.05$ using Statistical Package for the Social Sciences v. 22.0.

3. Results and discussion

3.1. Effects of cytokinins on shoot induction and proliferation

Different concentrations of BAP, TDZ, and Kin were used on Woody Plant Medium (WPM) to culture apical buds of *Ficus carica* var. Black Jack for the production of multiple shoots. Similar experiments on multiple shoot inductions were conducted by Rostami et al. [2], Danial et al. [10], Mantovani et al. [30] and Shekhawat et al. [31]. According to their results, the highest number of shoots was produced in media supplemented with BAP.

3.1.1. Effects of BAP on multiple shoot induction

Compared to TDZ and Kin, Woody Plant Medium (WPM) supplemented with BAP produced the highest number of shoots without browning of shoots and no callus formation on the growing cultures (Fig. 1; Table 1), with an exception of WPM + 15 μM BAP (Fig. 1D). Callus formation is one mechanism for wound healing in plants. Generally, wound-induced transcriptional factors are activated which programs the cells to produce callus on the wounded explant [32,33]. The emergence of a new shoot was observed after 2 weeks of culturing. The data obtained for shoot induction using WPM + BAP were highly significant than the data acquired for other media and concentrations (Table 1). Single plant growth regulator (PGR) treatment using BAP in WPM was successful for the induction of a maximum number of multiple shoots on the apical bud explants of *Ficus carica* var. Black Jack. In WPM supplemented with BAP (0 to 30 μM), it was evident that the concentration of 20 μM BAP was optimum to produce the highest number of healthy shoots ($37.80^{a} \pm 4.45$) on the apical bud explants of *Ficus carica* var. Back Jack (Fig. 1E; Table 1). Overall BAP is considered to be the most effective for shoot induction and proliferation [20,31,34,35]. The height of the multiple shoots produced was observed to be equally significant in all the media (T1 to T19).

3.1.2. Effects of TDZ and Kin on multiple shoot induction

The shoot growth on WPM containing TDZ and Kin was stunted and showed browning of callus and shoots (Table 1). The maximum number of shoots obtained using WPM supplemented with 20 μM TDZ was $3.33^{c} \pm 0.32$ with an average height of approximately 2.83 cm per shoot (Fig. 2E; Table 1). In the cultures which induced callus formation, it was observed that the number of shoots is drastically reduced. Along with WPM + 15 μM BAP (media T4) (Fig. 1), callus formation was observed to be most prominent on WPM supplemented with different concentrations of TDZ (media T14 to T19) (Fig. 2A–G; Table 1). Media containing TDZ produced a browning callus formation around the growing explants. Callus formation on the leaf explants had resulted in enhancement of the shoot induction on some *Ficus carica* cultivars using TDZ [20]. However, in some cases, the formation of callus can cease the growing multiple shoots. The phytohormones are directed towards callus generation. It is reported that wound-induced calli act as pluripotent cells by activating the downregulation genes for the inhibition of plant cell cycles [36]. Thus, callus formation and stunted growth of multiple shoots were justified on the excised apical buds.

Cultures with Kin as PGRs produced many single multiple shoots, but the growth was stunted in all the different concentrations that were tested (Fig. 3; Table 1). The highest number of shoots observed on WPM supplemented with 25 μM Kin was $5.33^{a} \pm 0.75$ with an average height of approximately 2.63 cm per shoot (Fig. 3F; Table 1). Stunted and unhealthy growth patterns in the plant cultures can be attributed to the interdependency of cell division and expansion of the tissues which may cause a deficiency in the uptake of the plant hormone by the growing cultures [30], considering the previous results and role of cytokinins in the cell division, and shoot induction and proliferation. Hence it was established that the BAP proved to be optimum for producing multiple shoots on the apical bud explants of *Ficus carica* var. Black Jack.

3.1.3. Effects of different concentrations of BAP on multiple shoot proliferation

A significantly higher number of multiple shoot induction and proliferation was obtained using WPM supplemented with

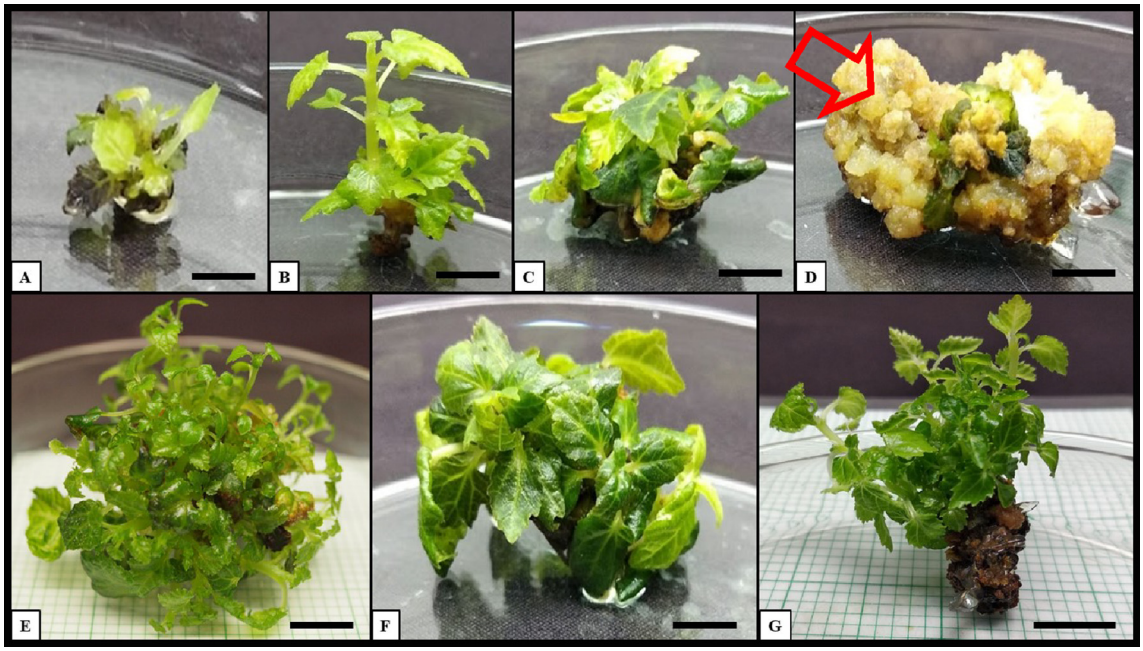

Fig. 1. Effects of WPM supplemented with different concentrations of BAP (T1–T7) on multiple shoot induction on apical buds of *Ficus carica* var. Black Jack after 20 weeks (S5). (A) 0 μM BAP, (B) 5 μM BAP, (C) 10 μM BAP, (D) 15 μM BAP, (E) 20 μM BAP, (F) 25 μM BAP, (G) 30 μM BAP. Scale bar represents 1 cm.

Table 1
Effects of different concentrations of BAP, TDZ, and Kin on the production of multiple shoots, mean height, percentage of callus formation, and browning of cultures of *Ficus carica* var. Black Jack.

Treatment	BAP (μM)	Kinetin (Kin) (μM)	TDZ (μM)	Number of shoots	Survival (%)	Height (cm)	Callus (%)	Browning (%)
T1 (Control)	0	0	0	$1.50^{bc} \pm 0.22$	100	$2.47^{a} \pm 0.94$	0	0
T2	5	–	–	$1.80^{bc} \pm 0.25$	100	$2.71^{a} \pm 0.11$	0	0
T3	10	–	–	$3.00^{bc} \pm 0.21$	100	$2.63^{a} \pm 0.11$	0	0
T4	15	–	–	$0.00^{c} \pm 0.00$	0	$0.00^{b} \pm 0.00$	100	–
T5	20	–	–	$37.80^{a} \pm 4.45$	100	$2.53^{a} \pm 0.15$	0	0
T6	25	–	–	$6.00^{b} \pm 0.54$	100	$2.68^{a} \pm 0.13$	0	0
T7	30	–	–	$3.50^{bc} \pm 0.43$	100	$2.67^{a} \pm 0.16$	0	0
T8	–	5	–	$2.53^{c} \pm 0.27$	100	$1.89^{d} \pm 0.89$	0	0
T9	–	10	–	$2.40^{c} \pm 0.29$	100	$2.07^{cd} \pm 0.14$	0	33.33
T10	–	15	–	$3.26^{c} \pm 0.32$	100	$2.56^{abc} \pm 0.13$	0	0
T11	–	20	–	$4.73^{ab} \pm 0.54$	100	$2.71^{abc} \pm 0.10$	0	40
T12	–	25	–	$5.33^{b} \pm 0.75$	100	$2.63^{abc} \pm 0.17$	0	50
T13	–	30	–	$3.53^{bc} \pm 0.52$	100	$2.57^{abc} \pm 0.17$	0	50
T14	–	–	5	$3.13^{c} \pm 0.50$	100	$2.27^{cde} \pm 0.14$	0	0
T15	–	–	10	$2.93^{c} \pm 0.37$	100	$2.99^{a} \pm 0.15$	26.67	50
T16	–	–	15	$2.93^{c} \pm 0.47$	100	$2.81^{ab} \pm 0.12$	53.33	66.67
T17	–	–	20	$3.33^{c} \pm 0.32$	100	$2.41^{bcd} \pm 0.15$	20	75
T18	–	–	25	$2.33^{c} \pm 0.21$	100	$2.83^{ab} \pm 0.17$	46.67	50
T19	–	–	30	$3.2^{c} \pm 0.43$	100	$2.65^{abc} \pm 0.12$	26.67	75

Means followed by the same letter within a column were not significantly different using Duncan's test ($p \geq 0.05$).

different concentrations of BAP (Table 1 and Table 2 respectively). The highest number of shoots (proliferation) was also obtained from the media supplemented with 20 μM BAP (medium number M5) with an average of 41.20 shoots with a 100% survival rate. The proliferating shoots showed 0% browning (Fig. 4E; Table 2) when grown on WPM supplemented with different concentrations of BAP. Therefore, the results prove that WPM medium containing BAP was the most suitable medium for the production of multiple shoots on *Ficus carica* var. Black Jack. Results have shown that 20 μM BAP in WPM produced the highest number of shoot induction and proliferation. Thus, BAP is a strong cytokinin that has proven to be very efficient for the production of a higher number of multiple shoots in *Ficus carica* species [10,19,37].

3.1.4. Carryover effects of the growth media

The highest number of multiple shoot induction (37.80^{a}) was observed using WPM with 20 μM BAP (T5 medium; Table 1). It can also be observed that the same medium (M5 medium, WPM + 20 μM BAP), could produce an optimal proliferation of the multiple shoots (41.20^{a}). This phenomenon can be explained by the carryover effects of growth media. The carryover effects entail that the growing cultures can survive in either the same media or PGR free media post multiple subculture cycles. Due to the subculture cycles, the dependency of *in vitro* cultures on the exogenous cytokinins is reduced. It is also a well-known fact that BAP has a significant carryover effect for *in vitro* growing cultures [38,39].

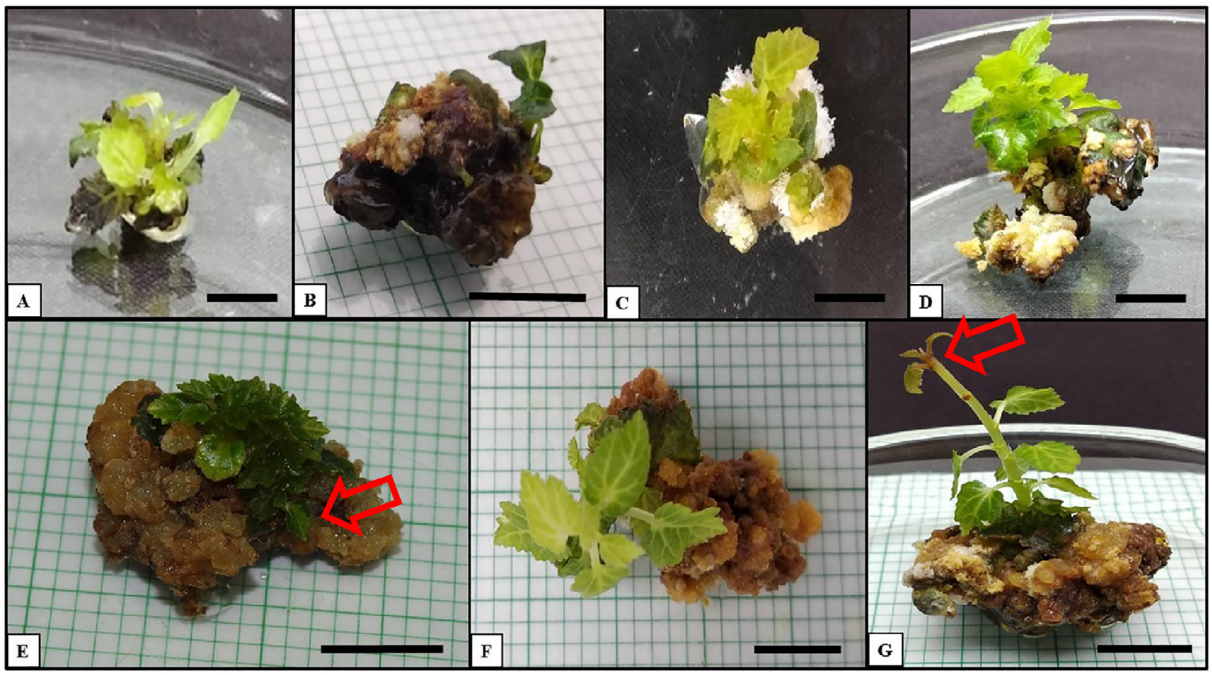

Fig. 2. Effects of WPM supplemented with different concentrations of TDZ (T1, and T8–T13) on multiple shoot induction on apical buds of *Ficus carica* var. Black Jack after 20 weeks (S5). (A) 0 μM TDZ, (B) 5 μM TDZ, (C) 10 μM TDZ, (D) 15 μM TDZ, (E) 20 μM TDZ, (F) 25 μM TDZ and (G) 30 μM TDZ. Scale bar represents 1 cm.

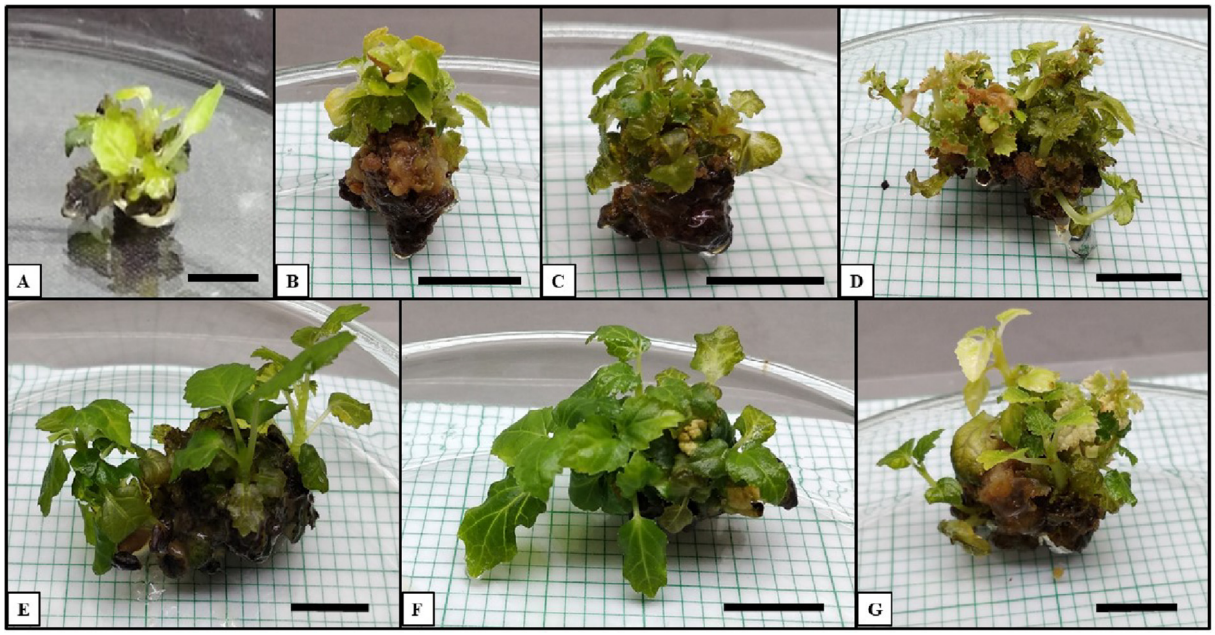

Fig. 3. Effects of WPM supplemented with different concentrations of Kinetin (T1, and T14–T19) on multiple shoot induction on apical buds of *Ficus carica* var. Black Jack after 20 weeks (S5). (A) 0 μM KIN, (B) 5 μM KIN, (C) 10 μM KIN, (D) 15 μM KIN, (E) 20 μM KIN, (F) 25 μM KIN and (G) 30 μM KIN. Scale bar represents 1 cm.

The concentration of BAP is retained in every subculture cycle and therefore the *in vitro* multiple shoots induced in WPM supplemented with 20 μM BAP. An analysis for the most effective PGR in WPM for the *in vitro* growth of *Corema album*, showed that the cultures which were initially grown in semi-solid media and then transferred to the temporary immersion bioreactor survived and rooted successfully. More than double the multiplication rate for the growing cultures was observed and was attributed to the carryover effects of the growth media [40]. The data obtained in a study conducted by Al-Shomali et al. [17], showed similar carryover effects for *in vitro* cultures of the common fig growing on three different media.

3.2. Induction of in vitro roots and acclimatization

Rooting in different varieties of *Ficus carica* has already been established using different combinations of NAA and IBA. Studies have shown that a combination of cytokinins and auxins is used in the growth media to enhance the shoot induction [19,20].

Table 2
Effects of different concentrations of BAP on the proliferation of multiple shoots using single shoots of *Ficus carica* var. Black Jack.

Treatment	BAP (μM)	Number of Shoots	Height	Survival (%)	Browning (%)
M1 (Control)	0	$1.40^{d} \pm 0.52$	$0.81^{c} \pm 0.27$	40	0
M2	5	$6.67^{cd} \pm 0.99$	$2.45^{ab} \pm 0.34$	80	0
M3	10	$10.53^{c} \pm 1.31$	$2.32^{ab} \pm 0.26$	86.67	0
M4	15	$18.73^{b} \pm 0.76$	$2.65^{a} \pm 0.12$	100	0
M5	20	$41.20^{a} \pm 2.48$	$3.21^{a} \pm 0.15$	100	0
M6	25	$19.53^{b} \pm 2.91$	$2.41^{ab} \pm 0.35$	90	0
M7	30	$10.00^{c} \pm 3.21$	$1.37^{bc} \pm 0.35$	76.67	0

Means followed by the same letter within a column were not significantly different using Duncan's test ($p \geq 0.05$).

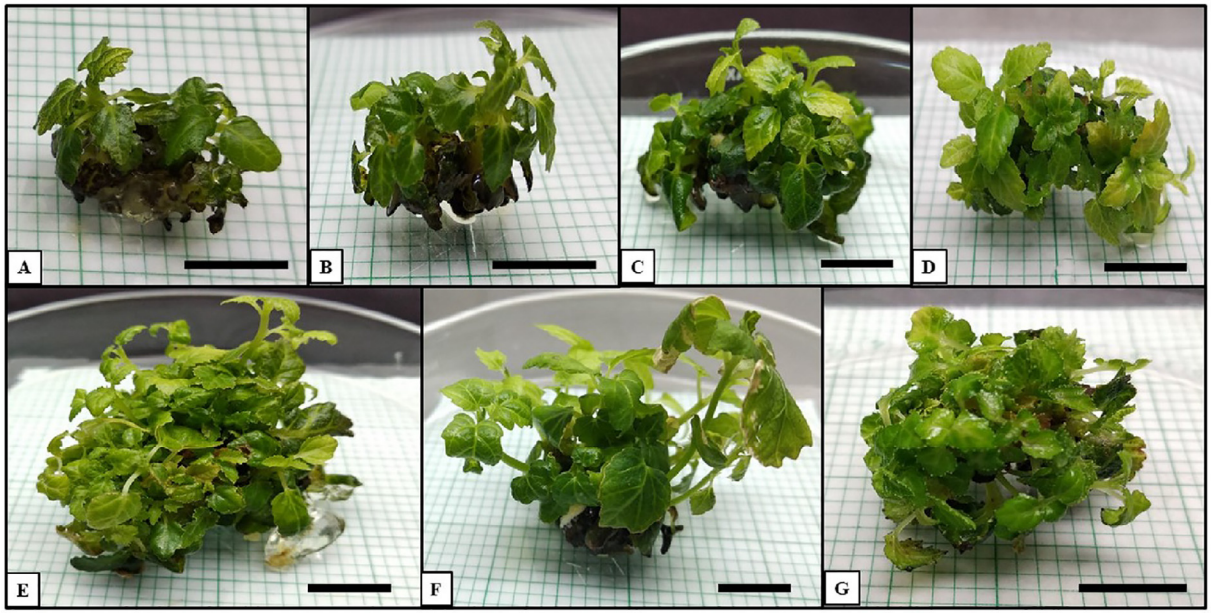

Fig. 4. Effects of WPM supplemented with different concentrations of BAP on the proliferation of shoots of *Ficus carica* var. Black Jack. (A) 0 μM BAP, (B) 5 μM BAP, (C) 10 μM BAP, (D) 15 μM BAP, (E) 20 μM BAP, (F) 25 μM BAP and (G) 30 μM BAP. Scale bar represents 1 cm.

3.2.1. Effects of IAA and IBA on root induction

Shoots with an average height of approximately 3.21 cm were transferred to the rooting medium (WPM) with different concentrations of IAA and IBA (Table 3). Fig. 5 shows the formation of roots on the *in vitro* generated shoots of *Ficus carica* var. Black Jack. The control medium T1 which was devoid of any growth regulator produced a slightly less percentage of roots on the shoots (86.67%), whereas 90 to 100% root induction was observed in WPM supplemented with different concentrations of IAA and IBA (Table 3). Although roots were observed on the WPM containing IBA, the number was significantly low. Overall, the roots were observed to be unhealthy (Fig. 5; Table 3). These results are in contrast to many other published reports, whereby IBA was used as a prime auxin that produced the highest number of roots on different plants [25,41,42].

The highest number of roots were generated on medium R5 (WPM + 20 μM BAP + 8 μM IAA) with an average of 4.33 number of roots per shoot with 100% successful root induction (Fig. 5; Table 3). The success of root induction using IAA in WPM can be attributed to the positive correlation between IAA and root formation [43].

Table 3
Effects of different concentrations of IAA and IBA on the induction of roots, on the single shoots of *Ficus carica* var. Black Jack.

Treatment	BAP (μM)	IAA (μM)	IBA (μM)	Number of Roots	Rooted plantlets (%)
R1 (Control)	0	0	0	$1.20^{b} \pm 0.17$	86.67
R2	20	2	–	$1.13^{b} \pm 0.13$	96.67
R3	20	4	–	$1.33^{b} \pm 0.19$	96.67
R4	20	6	–	$1.87^{b} \pm 0.17$	100
R5	20	8	–	$4.33^{a} \pm 0.64$	100
R6	20	10	–	$1.80^{b} \pm 0.22$	100
R7	20	–	2	$1.53^{b} \pm 0.17$	100
R8	20	–	4	$1.73^{b} \pm 0.18$	100
R9	20	–	6	$1.33^{b} \pm 0.19$	93.33
R10	20	–	8	$1.47^{b} \pm 0.24$	90
R11	20	–	10	$1.27^{b} \pm 0.23$	90

Means followed by the same letter within a column were not significantly different using Duncan's test ($p \geq 0.05$).

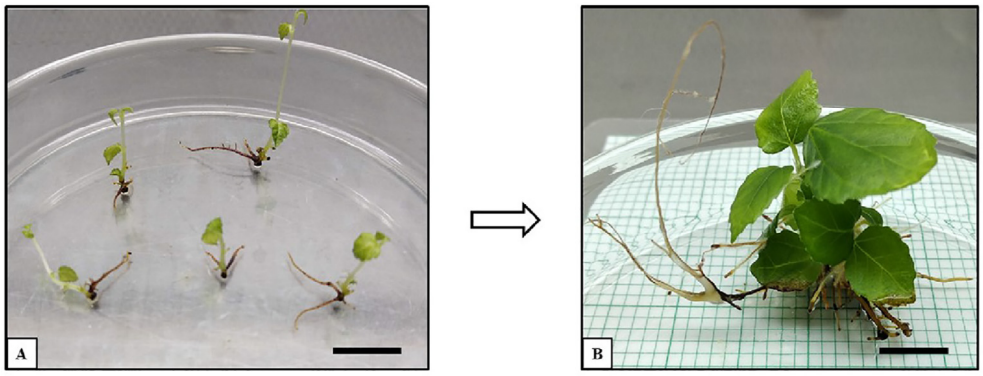

Fig. 5. Effects of WPM supplemented with 20 μM BAP and 8 μM IAA on induction of roots from the single shoot of *Ficus carica* var. Black Jack. (A) Roots induced after 2 weeks on WPM + 20 μM BAP + 8 μM IAA and (B) Rooted plantlet after four weeks of culture. Scale bar represents 1 cm.

3.2.2. Acclimatization

Anatomically the plantlets are devoid of most of the cell structures which can be generally found in the plants growing in natural environments. Therefore, it is essential to ensure a proper hardening technique that will adhere to the plantlet's fragile condition. A general acclimatization protocol will include detailed steps which will ensure that the plantlets follow a continuous change in the environment. The process of adaptation of plantlets to the soil conditions after transferring them from the solid media is highly significant [34,44]. Rooted plantlets were used to successfully acclimatise the plants to the outside environment. *In vitro* plants of *Ficus carica* var. Black Jack was successfully acclimatised using a systematic protocol and after four weeks of *ex vitro* acclimatization, the plants were transferred to larger pots for further *ex vitro* cultivation. Fig. 6 displays the successfully acclimatised plants after eight weeks of acclimatization in the *ex vitro* conditions.

Fig. 6. *Ficus carica* var. Black Jack plants after acclimatization using sterile Biochar soil. Eight weeks post *ex vitro* acclimatization. Scale bar represents 5 cm.

4. Conclusions

Multiple shoots were successfully induced on apical bud explants of *Ficus carica* var. Black Jack. The optimum medium for multiple shoot induction and proliferation of *Ficus carica* var. Black Jack was obtained using WPM supplemented with 20 μM BAP (Media T5 and M5 respectively). For the root induction on the single shoots acquired from shoot proliferation of *Ficus carica* var. Black Jack, WPM + 20 μM BAP + 8 μM IAA (Media R5) was optimum. The highest number of root induction was obtained using this medium. Rooted plants were further used for *ex vitro* acclimatization using sterile Biochar soil.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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