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Research Paper

Multiple Shoots Production from Shoot Tips of Fig Tree (*Ficus carica* L.) and Callus Induction from Leaf Segments

Gharbia H. Danial[1, *], Diaa A. Ibrahim[1], Sazan A. Brkat[1] and Belan M. Khalil[1]

[1] Scientific Research Center, Faculty of Science, University of Duhok, Iraq

* Corresponding author, e-mail: (gha.2008@yahoo.com)

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Abstract: *Cultivated fig (Ficus carica L.) is commonly grown in Kurdistan Region of Iraq for garden specimen as well as fruit tree with some propagation difficulties. For rapid clonal propagation of this plant, the shoot apices from actively growing adult plants, were excised and cultured on MS medium supplemented with various combinations of growth regulators to induce shoot proliferation and subsequent rooting. Following 4 weeks of incubation, the shoot tips grew into 4cm tall shoots in a medium supplemented with 0.5mg/l BAP. The effects of the cytokinins BAP and kinetin were tested for their ability to induce shoot multiplication, while the auxins IBA and NAA were tested for rooting, leaf disc explants of fig were used as a starting material for callus induction. Explants were cultured on MS medium having different concentrations of plant regulators (kinetin and 2, 4-D). The results revealed that the optimal concentration for shoot multiplication ranged between 0.5 and 3.0 mg/l BAP. No beneficial effect was obtained from inclusion of Kinetin in the culture medium. On the other hand, rooting of individual shoots was attained in a medium enriched with 0.5 mg/l IBA and NAA. The best medium for callus induction shown in the medium supplemented with 0.4 mg/l Kinetin and 4.0 mg/l 2, 4-D. The gradually acclimatized plants were successfully transferred to the out-air conditions and a high survival rate (95%) was recorded finally the successfully protocol for in vitro culture of Ficus carica has also supported the research on transgenic plants obtained by genetic transformation.*

Keywords: Fig, *Ficus carica*, micropropagation, woody plant, leaf callus.

1. Introduction:

Ficus carica, the well known commercial fig, a member of Moraceae family is an important tree in many rural areas, it is native to Western Asia but distributed by man throughout the Mediterranean region (Morton, 1987), Fig was one of the first plants ever to be cultivated by humans, it is a woody plant with highly varied forms, bushes, shrubs, small trees, very tall and large trees. Plants are usually monoecious with small flowers which they are all female and need no pollination in common fig, without petals and nectarines (Wang and Charles, 1991). The fruit color varies from yellowish-green to coppery, bronze, or dark-purple. The fruit is well known for its nutritive value and is consumed either fresh or dry worldwide and for their mild laxative activity and high alkalinity. Moreover, active ingredients derived from fig are used in various drug preparations. (Kirtikar and Basu, 1986). Fig trees are propagated via cutting of mature wood or grafts as a result of seeds are nonviable, this kind of vegetative propagation insures uniformity, relatively low multiplication rates because those materials can be obtained only from upright branches which results in poor rooting (Kumar et al., 1998). Therefore, multiplication by tissue culture techniques could be advantageous due to the production of high quality disease free, true-to-type plants independent of seasonal and other environmental conditions in a comparatively smaller space (Debergh and Zimmerman, 1991).

The most important technique of micropropagation reported by various researchers using cultures of apical meristems and axillary buds to regenerate multiple shoots (Fraguas *et al*. 2004; Moon Kim *et al*. 2007; Al khaybari 2008; Flores- Mora *et al*. 2009; Soliman *et al*. 2010). Other important technique of Fig studies shown in callus induction and biochemical assessment of active compounds in fig calli induction from different parts in the plant (Nassar and Newbury 1987; Cormier *et al*., 1989). On the other hand, shoot was successfully regenerated from the axillary bud of mature trees (Deshpande *et al*. 1998), from apical bud (Kumar *et al*., 1998) and from the calli of stem segment (Jaiswal and Narayan 1985).

The main objective in this study is to establish an *in vitro* shoot proliferation protocol for *Ficus carica* which is an important fruit in Kurdistan Region of Iraq. The result may lead to the develops a specific protocol for rapid shoot proliferation needed to be used for sustaining fig plantation in Iraq from genetically uniform and healthy plants that can be used for different objectives, in addition to identify the best medium for callus induction from the leaf segments.

2. Materials and Methods:

Healthy growing shoots collected from, Duhok city, Kurdistan Region of Iraq in April, 2011. The shoots were thoroughly washed using tap water to remove dirts and dusts, followed by surface disinfestation by immersing in a mixture of 5% NaOCl solution containing few drops of Tween-20 surfactant. A gentile vacuum was applied for 15 min. to dislodge the air bubbles possibly captured within the tissues. Under aseptic conditions, the disinfestant was discarded and the shoots were rinsed 3 times with autoclaved deionized water. The shoot apex, consisting of apical meristeme, leaf primordial were excised and transferred to culture vessel containing(25 ml) nutrient medium.

Murashige and Skoog (1962) was the base medium used in all protocols supplemented with the followings (in mg/l): sucrose (30,000), inositol (100), thiamine HCl (0.4), BAP (0.5) and glycine (0.5). pH was adjusted to 5.7± 0.1 with 1N NaOH or HCl, prior to the addition of agar (7, 000). The medium was brought up to the final volume, then dispensed at 25 ml rates into 250 ml Mason jars and capped with colorless PVP covers and fitted with rubber bunds. The medium was autoclaved for 15 min at 121 C° and 1.04 kg/cm².

Nodal explants (1 cm) with two buds were excised from developed shoots and cultured on the same media for the culture initiation to induce shoot multiplication. The first experiment include five concentration (0.0, 0.5, 1.0, 2.0, and 3.0 mg/l) of two kinds of cytokinin BAP (Benzylamino purine) and kinetin. After 6 weeks the number and length of newly initiated shoots were recorded.

The second experiment was similar to the first one but consisted of using two kinds of auxins IBA (indole -1- butyric acid) and NAA (naphthalene -1- acetic acid) for root induction at the same levels of cytokinin. Data regarding the roots length and numbers were recorded after 6 weeks of incubation.

Excised leaf discs ($1\times1cm^2$) of 30 day old *in vitro* plants were used for callus induction in the third experiment using the basal media supplement with a combination of different concentration (0.0, 0.2, 2.0, 4.0 and 0.2, 0.4, 2.0, 4.0) of both kinetin and 2,4-D. All the cultures were transferred to growth room for a period of almost 4-6 weeks.

The cultures were maintained in growth room at 25± 1°C under16 hours photoperiod with light intensity of 1000 Lux cool white light, followed by 8 hours of darkness. Data was analyzed to determine the least significant differences according to SAS (2001).

Successfully rooted plantlets were removed from their containers, followed by washing the roots then immersed in Benlate fungicide (0.1% for 10 minutes).then transferred to pots containing a steam sterilized soil mix (peatmoss+ loam+ Styrofoam 1:1:0.5, v:v:v) under tightly controlled atmosphere of the greenhouse.

3. Results and Discussion:

In order to determine proper sterilization procedure, shoot tips were successfully sterilized by immersion in 3:1 chlorax: water (vol.: vol.) coupled with slight vacuum, followed by washing the samples three times in deionized distilled water and surface sterilization was efficient in eliminating various pathogens such as bacteria and fungi, as well as prevention of browning of explants during fig tissue cultures by daily transfer to fresh media.

Proliferation of the cultured shoot apex started through the first two weeks of incubation in the culture room. Single shoots developed from the cultured apex reached 3- 4 cm tall during four weeks of incubation. No abnormalities were observed in the newly initiated shoots, and no callus was associated with their development (Fig. 5, A and B). The initiation stage was thus terminated, and the new shoots were transferred to the multiplication stage. The shoots were divided into small nodal segments and cultured on fresh medium supplemented with various concentrations of BAP or kinetin.

Enhanced axillary shoot proliferation was observed in the shoot segments after 4-6 weeks in the media supplemented with BAP, however the number of new shoots varied according to BAP concentration (Figure 1). The number of branches per explant was highly influenced by the addition of BAP to the nutrient medium. The highest number of branches (5.608) was achieved from the addition of 3.0 mg/l BAP (Fig. 5. C and D). While the lowest number of branches per explant (1.083) was recorded in the control treatment. A reverse correlation can be found between the number of branches and the mean length of branches, since BAP caused a remarkable reduction in the mean length of branches as compared to the control treatment from 1.567 cm (control) to only 0.908 cm (4.0 mg/l BAP). This reduction in length may be attributable to the competition on nutrient media and space in cultures received high BAP concentration. The highest number of leaves per explant was recorded for the (3.0 mg/l BAP) treatment (11.573), which was significantly reduced to only 3.998 leaves/ explant in the control treatment.

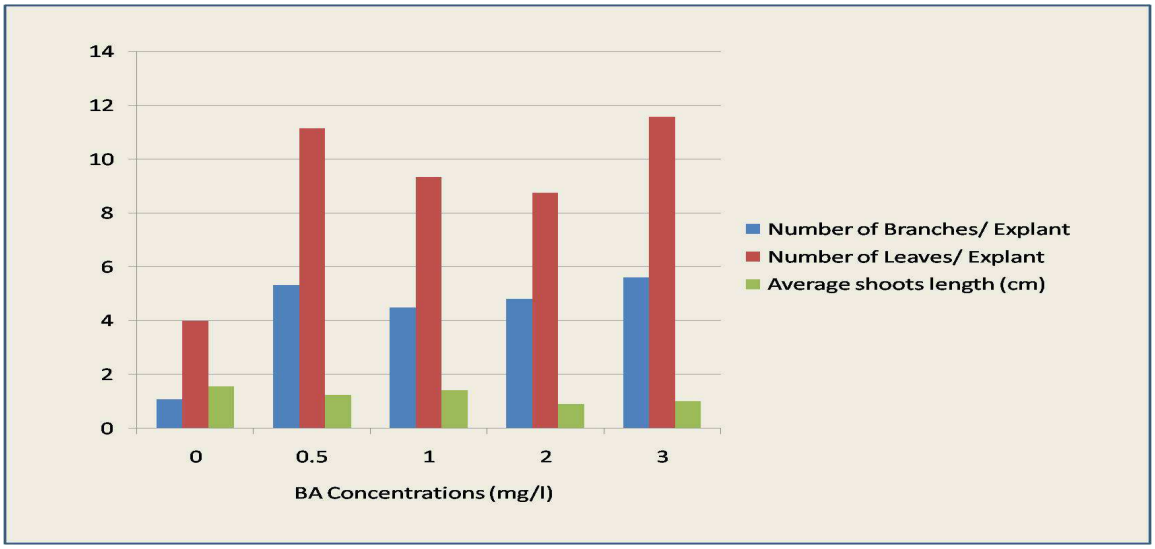


Figure (1): Effect of different BAP concentrations on fig plantlets multiplication after six weeks in culture

The reasons behind the positive role of BAP on multiplication stage might be due to cytokinins great role in releasing lateral buds from the dominance of terminal buds without need to remove the apical bud by promoting the formation of xylem tissues of buds which will facilitate the transformation of water and nutrients leading to lateral bud growth (Mohammed and Al-Younis, 1991) as well as, the important role of cytokinins in increasing the synthesis of RNA, proteins and enzymes inside the cell which enhance bud growth as well (Al-Rifae'e and Al-Shobaki, 2002; Toma and Rasheed, 2012).

The effect of different concentration of kinetin on fig plant shoots multiplication is shown in (Figure 2). Adding 2.0 mg/l kinetin gave highest number of branches per explant (1.415), highest average shoots length (1.523) and highest number of leaves/explants (4.915), but did not show significant differences when compared to the control treatments. The kinetin showed a less stimulatory effect on shooting when compared to BAP. This can be explained on the basis of two double bonds in BAP structure, compared to single bond in kinetin molecule (Robins *et. al*., 1986).

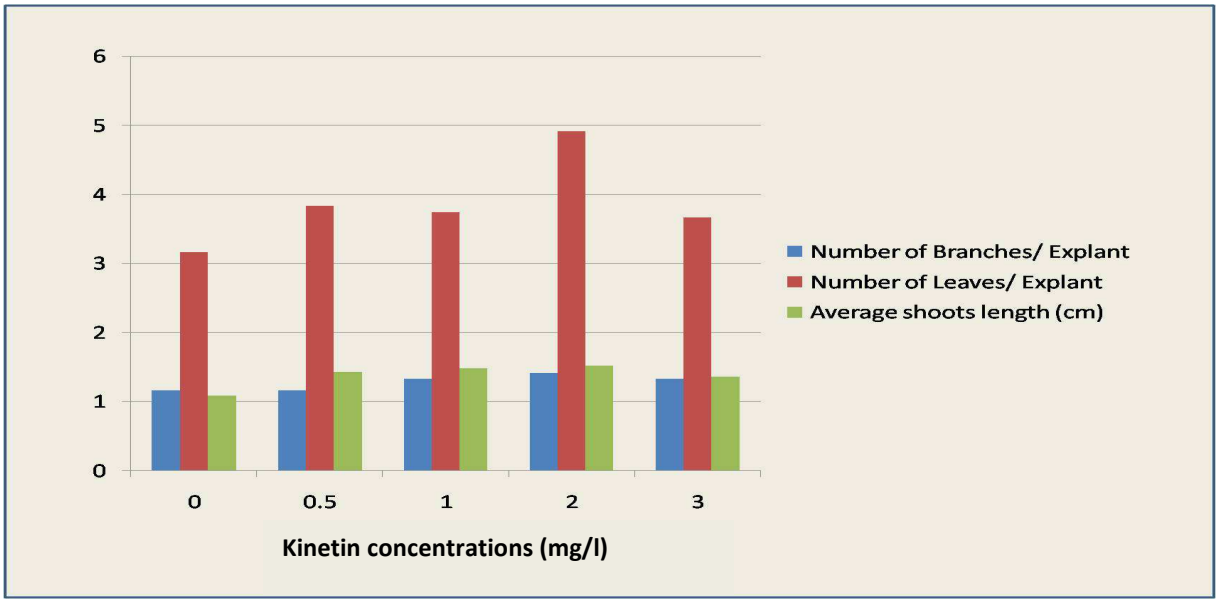


Figure (2): Effect of different Kinetin concentrations on fig plantlets multiplication after six weeks in culture

The results revealed that the two tested auxins (NAA and IBA) stimulated root initiation in *Ficus carica* L., however the extend of rooting varied according to auxin type and concentration. NAA was the most effective auxin, followed by IBA.

Figure: 3 shows that the optimal concentration of NAA to induce rooting is 0.5 mg/l, where 2.833 roots as an average were initiated at each shoot (Fig.5. E and F), and there was no significant differences when compared with control treatment. A comparable number of roots were also developed at 1.0 and 3.0 mg/l NAA level, however, no significant differences where observed between them. Other NAA concentration (i.e. 2.0 mg/l) also stimulated rooting to a lesser extent and significant differences were comparable to the control and other treatment. As for root length, it was observed that an inverse relation was existed between NAA concentration and root length. Best root length was (8.218 cm) achieved at control treatment; this increase in length over the NAA treatment was not significant when comparable to the NAA treatment except 2.0 mg/l NAA was (1.020 cm). (Table3).

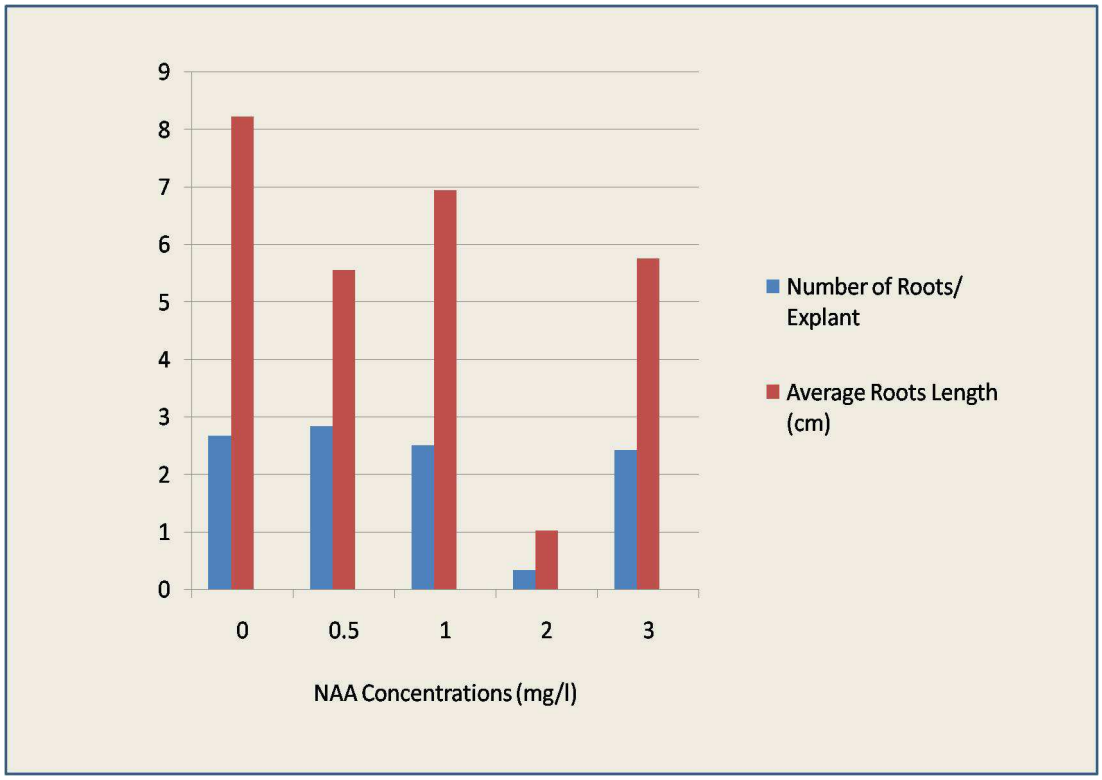


Figure (3): Effect of different NAA concentrations on fig plantlets rooting after six weeks in culture

Figure 4 shows significantly higher number of roots per explant (2.33) was recorded for those explants grown on medium supplemented with 0.5 mg/l IBA as compared with those grown on medium contain 1.0, 2.0 and 3.0 mg/l IBA but this increase was not significant when compared to the control treatment. The mean length of roots was not significantly affected by using IBA. Control treatment which gave the longest mean length of root (2.750) was significantly different with both 2.0 and 3.0 mg/l and not significant with the 0.5 and 1.0 mg/l IBA treatment.

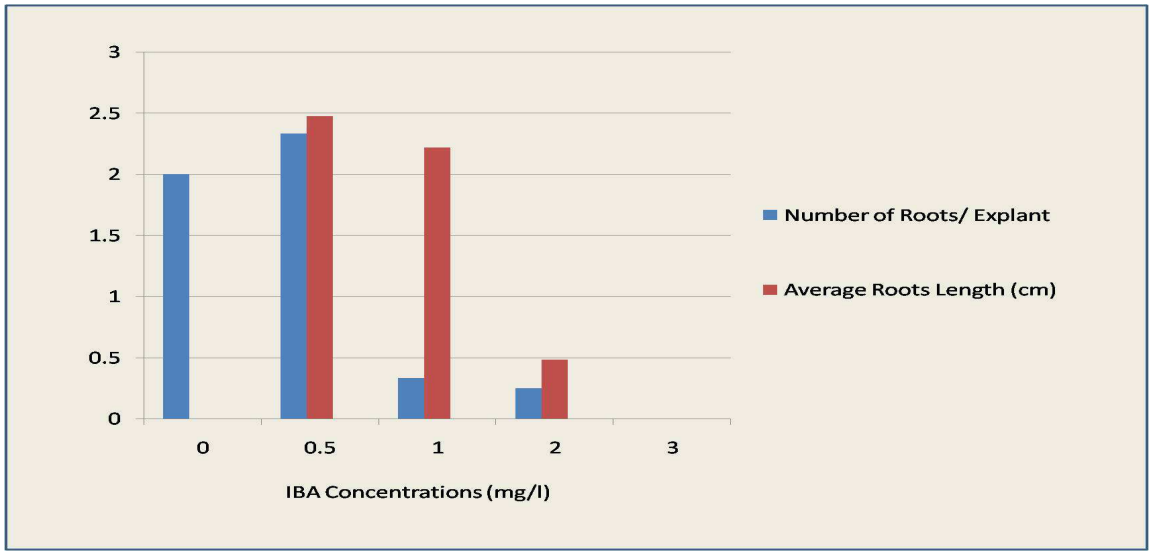


Figure (4): Effect of different IBA concentrations on fig plantlets rooting after six weeks of culture

Data in figure (3 and 4) showed the results of this experiment. The use of different concentrations of NAA or IBA was non affective in raising rooting parameters as compared with auxin-free medium (control). This indicates the high endogenous auxin content of shoots which was sufficient for rooting.

4. Callus Induction of *F. carica* L.

All tested media had ability to form callus from the leaf segments Table 1. The data indicated that The highest survival percentage (100%) was obtained on MS medium supplemented with 0.2 mg/l kinetin with 2.0 mg/l 2,4-D and 0.4 mg/l kinetin with 4.0 mg/ l 2,4-D , whereas the lowest one found in 2.0 mg/l kinetin with 0.2mg/l 2,4-D (16.67)% which was significant different when was compared with other treatment. On the other hand the high callus weight (1.823 g) recorded at 0.4 mg/l kinetin with 4.0 mg/l 2,4-D and significant difference when compared with other treatment, and low callus weight (0.533 g) recorded at 4mg/l kinetin with 0.4 mg/l 2.4-D.

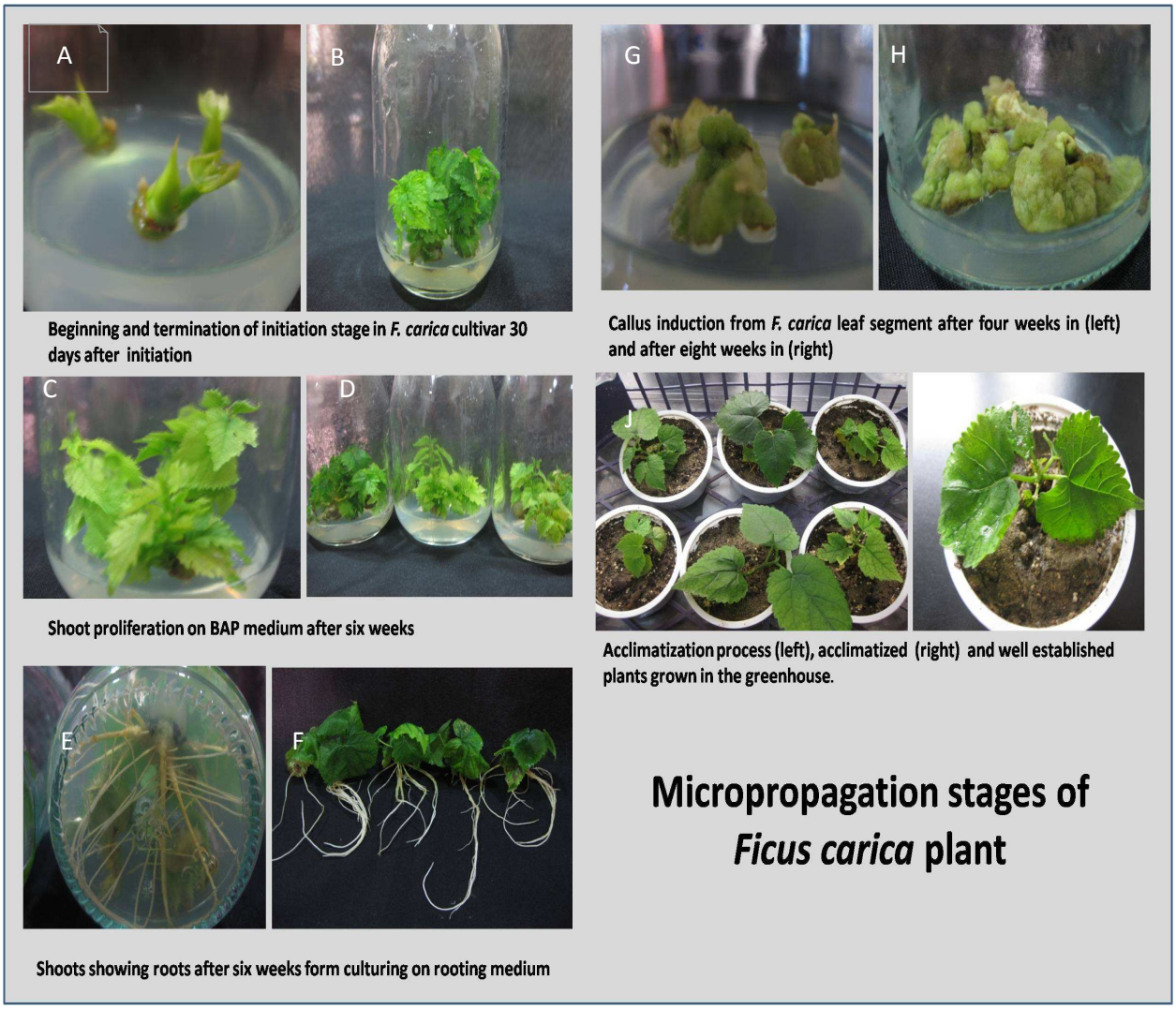
Table (1): Effect of Kinetin and 2, 4-D combination in callus initiation from fig leaves explants after eight weeks in culture

concentrations (mg/l)	Callus Initiation Rate (%)	Callus Weight (g)
0.0 mg kin+ 2mg 2,4 D	83.34 a	1.063 b
0.2 mg kin+ 2mg 2,4 D	100.00 a	1.210 b
2 mg kin+ 0.2mg 2,4 D	16.67 b	0.055 d
0.4 mg kin+ 4mg 2,4 D	100.00 a	1.823 a
4 mg kin+ 0.4mg 2,4 D	83.34 a	0.533 c

*Different letters within columns represent significant differences according to Duncan's multiple range test at 5% level.

In all treatments, it was found that the combination of 2, 4-D and kinetin in various concentrations in callus induction for leaf segments had varying effect in callus induction and weight. Comparison between all treatment applied showed that 2, 4-D is essential for callus induction and addition of kinetin in certain concentration will increase the callus growth in leaf culture. This whole outcome showed that 2, 4-D was essential for callus induction and kinetin was useful to promote callus growth (Joyner *et al* 2010; Dalila *et al* 2013).

In the present study callus formed from leaf segments cultured in a combination of BA with NAA and BA with Kinetin. No shoot morphogenesis occurred from explants cultured on any test combinations of auxins and cytokinins (data not shown). A number of well developed plantlets of root and shoot systems were successfully hardened and acclimatized using a grade mixture consisting of peatmoss+ loam+ styrofoam (1: 1: 0.5). The hardened plants (Fig. 5, J and K) were established in soil during (3) weeks.



Figur 5: Micropropagation stages of *Ficus carica* plant

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