

DOI: <https://doi.org/10.24297/jaa.v10i0.8160>**Micropropagation and Conservation of Fig (*Ficus Carica* L.)**Mohamad A. Shatnawi<sup>1\*</sup>, Rida A. Shibli<sup>2</sup>, Wesam G. Shahrour<sup>1</sup> Tamara S. Al-Qudah<sup>3</sup> and Taleb Abu-Zahra<sup>1</sup><sup>1</sup>Biotechnology Department. Faculty of Agricultural Technology. Al-Balqa' Applied University, Al-Salt 19117, Jordan.<sup>2</sup>Department of Horticulture and Agronomy, Faculty of Agriculture. University of Jordan, Amman, Jordan.<sup>3</sup>Hamdi Mango Center for Scientific Research University of Jordan, Amman, Jordan \*

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**Abstract**

An efficient protocol is outlined for rapid and mass micropropagation of *Ficus carica* L. (fig). Shoot tips (5 mm) were obtained from mother plants stock grown on half strength Murashige and Skoog (½ MS) medium with the addition of 30 g/L sucrose. For shoot multiplication Benzyl amino purine (BAP) and kinetin produced differences number of new shoot per plant and shoot height. BAP at 0.4 mg/L in combination with 0.2 mg/L indole-3-butyric-acid (IBA) produce maximum *in vitro* propagation rate, with 4.2 shoots per ex-plant. Root initiation was experimented on MS medium containing different concentrations of mg/L, IBA, IAA (Indole-3-acetic-acid) (IAA) or Naphthalene acetic acid (NAA). Highest number of root (4.3) was resulted when 1.5 mg/L IBA was used. After acclimatization in a mixture of (1 soil: 1 perlite: 1 peat) survival rate of 80% was achieved. For *in vitro* conservation of *F. carica* was experimented as microshoots were stored for 40 weeks on MS medium containing different sucrose concentration. Medium supplemented with 3% sucrose gave the highest regrowth (89%) at 24 ± 2 °C. Culture grew slowly when transferred to new fresh medium after the storage periods.

**Keywords:** Conservation, Germplasm, *Ficus Carica*, Micropropagation**Introduction**

Fig (*Ficus carica* L.) belonging to the order of Urticales and the family of Moraceae, it can be eaten fresh, dried or jam, it is a deciduous plant native to southwest Asia and the eastern Mediterranean region (Zohary and Spiegel-Roy, 1975). Figs are good source of dietary fiber and minerals, rich source of amino acids, antioxidant and vitamins and its free of sodium and are fat and cholesterol-free (Chessa, 1997, Soloman et al., 2006). Guarrera (2005) indicated that *F. carica* have been used as laxative, antispasmodic, respiratory, anti-inflammatory remedies and cardiovascular, as well as, cancer preventive and cancer therapeutic (Lansky et al., 2008). *F. carica* cuttings produced poor rooting percentage because those materials can be obtained only from upright branches, while seed are non-viable (Kumar et al., 1998). Due to the above-mentioned problems, to face these challenges *in vitro* propagation is the alternative method for multiplication of *F. carica* plants. To benefit from this technique to obtain mass and rapid propagation, thus *in vitro* techniques could be utilized.

Propagating plant using *in vitro* culture techniques has some advantages over conventional propagation methods, as it supplies with uniform plants throughout the year (Ebrahim et al., 2007, Shatnawi et al., 2010; 2011a,b; Shibli 1999; 2006; 2007). Micropropagation of *F. carica* can be carried out through *in vitro* shoot multiplication or by organogenesis methods provides large number of genetically true to type plant. Plant conserved under field condition methods suffers from biotic and abiotic stress (Arafah et al., 2007; Shibli et al., 2006; Subaih et al., 2007; Shatnawi et al., 2011a). *In vitro* conservation of plant material could offer practical means for germplasm preservation (Engelmann, 1997; Shibli et al., 2003; 2006). Conservation of *in vitro* plants are recommended to be used in germplasm conservation centers.

Microshoot propagation using meristem culture is another option for *F. carica* propagation (Murithi et al., 1982, Shibli et al., 1997, Demiralay et al., 1998; Kumar et al., 1998; Shatnawi et al., 2004). Yakushiji et al. (2003) have developed a method for inducing organogenesis from leaf explants of *F. carica*, but their frequency of adventitious bud differentiation has been low. A study by Qrunfleh et al. (2013) showed that different carbon sources showed significant differences in regrowth *in vitro* regarding number of newly formed shoots and their shoot lengths. On the other hand, Kumar et al. (1998) showed that simple micropropagation method for *F. carica* was developed on ½ MS medium containing 2.0 mg/L IAA and 0.2% activated charcoal when apical buds were used. A critical problem associated with *in vitro* culture of fig is the darkening or browning of the culture medium that can be toxic to plant growth which is attributed to the oxidation of phenol compounds that are excreted from the cut edges of explants and accumulate in that medium (Laukkanen et al. 1999).

A study by Demiralay et al. (1998) on *F. carica* showed that micropropagation (4.43 shoots/explant) was obtained on MS medium including 1.0 mg/L BA and 89 mg/L phloroglucinol. Nober & Romano (1998) enhanced rooting efficiency in *F. carica* by addition of 0.05% PVP in the culture medium containing 0.5 mg/L IBA. While Barbosa et al. (1992) enhanced *in vitro* shoot quality of *F. carica* on MS medium supplemented with 3.0 mg/L charcoal. Micropropagation of *F. carica* using *in vitro* techniques as initial source of plant material for *in vitro* conservation of *F. carica* has not been optimized up to date to date. Therefore, the present study provides simple, reliable micropropagation system for *F. carica* as initial step for medium-term *in vitro* conservation.

## **MATERIALS AND METHODS**

### ***Selection of explants:***

Shoots of *F. carica* (cv. Salti Kodari) were obtained from Hormart Al-Sahen Field station, Al-Salt, Jordan (latitude and longitude are 32.034201° N and 35.792968° E). From ten years old mother plants, shoot tips were selected and excised to 3-5 mm in length. Then shoot tips were dipped in 70% alcohol for thirty seconds and they were put in 4% NaOCl, together with few drops of Tween 20 for 20 min. Sterile shoot tips were rinsed three times with sterile deionized water under laminar flow cabinet.

### ***Culture conditions:***

Sterile shoot tips were grown on half strength Murashige & Skoog (½ MS) medium (1962) devoid of hormones including 30 g/L sucrose, pH was adjusted to 5.8 prior autoclaving, and 7 g/L agar agar was supplemented to the medium. Sixty ml media were dispensed in 250 mL flask. Explants were incubated at 24 ± 2 °C with a 16 h photoperiod and irradiance of 50 μmol m<sup>-2</sup>s<sup>-1</sup> supplied by cool white fluorescent lamps. After six weeks' growth, microshoots produced from these explants were cultured on medium including 0.2 mg/L kinetin and 0.01 mg/L indole butyric acid (IBA) with the addition of 1.0 g/L charcoal and then subcultured every six to seven weeks to obtain enough plant material free from phenols before experimentation.

### ***Effect of cytokinins:***

Developed microshoots from mother plants (15 mm in length) were then cultured on MS medium devoid of hormones, for two weeks before initiation shoot multiplication experiment. Then microshoots were cultured on MS medium containing benzyl amino purine (BAP), kinetin or zeatin at 0.0, 0.4, 0.8, 1.2, 1.6 or 2.0 mg/L, with or without 0.2 mg/L naphthalene acetic acid (NAA). Sixty mL of the medium were dispensed into 250 mL flask for each replicate. Each treatment replicates were repeated for twenty replicates. After six weeks, data were collected on number of shoots per initial explant and maximum shoot height.

### ***In vitro root formation:***

Microshoots (15 mm in length) were cultured on MS medium containing IBA, indole acetic acid (IAA) or NAA at 0.0, 0.3, 0.6, 1.2, 1.5 or 2.0 mg/L. Each treatment consisted of 20 replicates on sixty mL medium in 250 mL flask. Six weeks later, results were collected on shoot length, root number, root length, and rooting percentage.

#### **Effect of pH on microshoots growth:**

Microshoots 15 mm in length with two leaves were cultured on MS supplemented with 0.3 mg/L BAP; pH was adjusted 4, 4.5, 5.0, 5.5 or 6.0. After six weeks' growth period data were collected on shoot height and number of shoot per explant.

#### **Ex vitro acclimatization:**

Rooted *in vitro* plantlets were separated from the medium and washed gently with sterile distilled water at room temperature and then transferred to 9 x 9 x 9 in a mixture of (1 soil: 1 perlite: 1 peat) according to our previous report by Shatnawi et al. (2011).

#### **Medium term conservation and recovery of shoots:**

According to previous report by Shatnawi et al. (2011) to avoid any carry over effect of the cytokinin prior to the experiment, *in vitro* plantlets were subjected to MS medium hormone for two weeks. Microshoots of 15 mm length were cultured MS medium devoid hormone containing different concentration of sucrose as follow: 0, 30, 60, 90 or 120 g/L. The stored explants were incubated in the growth room with 16 h photoperiod ( $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) or under complete dark. Survival was recorded after 20 and 40 weeks of storage periods. Then after forty weeks' storage survived shoots were transferred to regrowth MS devoid hormones.

#### **Statistical analysis:**

Data were subjected to Analysis of variance (ANOVA) using STATISTICA (Stassoft 1995) and comparison of mean values were separated according to the least significant differences test (LSD) at  $p \leq 0.05$ .

## **RESULTS AND DISCUSSION**

#### **Clonal propagation:**

Phenol leakage from shoot basal-end was released on the medium. Repeated subculture was efficient to avoid availability of phenols compound. Therefore, activated charcoal (1.0 g/L) was added to the medium to get rid of phenol compound. This study showed that *in vitro* propagation of *F. carica* has the capability for producing large number of *in vitro* plantlet. Microshoots cultured on MS medium supplemented with different cytokinins (BAP, kinetin and zeatin) singly or in combination with 0.2 mg/L IBA resulted in varied responses with respect to shoot length and number of new shoots per ex-plant (Table 1). Of the three cytokinins tested, BAP was superior to kinetin and zeatin. Using 0.4 mg/L BAP found to be the most effective for formation of new shoots. While maximum shoot length (36.2-36.6 mm) was produced at 0.4 or 0.8 mg/L BAP supplemented with 0.2 mg/L IBA (Fig. 1). The results of the current study were similar to previous finding on *Stevia rebaudiana* (Shatnawi et al., 2011b), and *Syzygium francisii* (Shatnawi et al., 2004).

Shoot number (2.9) and shoot length (about 3.0 cm) was decreased using 2.0 mg/L BAP. Similar to our finding Hazara et al. (2002) on *F. carica* reported that BAP induce adventitious buds from fig plant tissue. Also Pontikis et al. (1986) obtained maximum shoot number on *F. carica* by the using of BAP. At 0.4 mg/L BAP and 0.2 mg/L IBA Number of new shoot (4.2) increased significantly (Table 1). Therefore, in the present study, enhancement of multiplication may be due to the interaction between BAP and IBA which lead to the ability to induce shoot formation. The use of kinetin did not improved proliferation. When using of kinetin, the maximums shoot number (1.2 shoots per ex-plant) was occurred with or without IBA. Supplemented kinetin in culture in combination with 0.2 mg/L IBA did not increased shoot proliferation. This may suggest that kinetin did not

play key role in shoot proliferation since it may not cause increased meristematic activity that leads to multiple shoot formation. When zeatin was used maximum proliferation (1.3-1.9 shoots per explant) could be reached (Table 1). The presences of IBA did not increase number of new developed shoots. Conversely, high zeatin concentrations cause morphological, physiological and anatomical, disorders on fig *in plant*. In this study with the presences of BAP, kinetin or zeatin root formation was diminished or inhibited; these results similar to previous finding in *F. carica* (Kumar et al., 1998, Fraquas et al., 2004).

#### **Root formation in vitro:**

In MS medium devoid auxins, fig microshoots failed to root. Better root formation was facilitated by the addition of IBA, NAA or IAA. However, roots were emerged at the base of shoots after two weeks. Both the concentration and the type of IBA, NAA or IAA used markedly influenced number of roots, root length and rooting percentage (Table 2). Maximum shoot length was produced using 0.9 mg/L IBA, while higher concentration has no influenced. However, maximum root number (4.3), and root length (29.6 mm) were produced on 1.5 mg/L IBA.

At low IBA concentration number of roots per explant was low and it induces thin roots (Table 2). Similar results were also presented by Shatnawi et al. (2007) in *Prunus avium*. Higher rooting percentage was obtained on media containing higher IBA, NAA or IAA. With the using of 1.2 mg/L IBA rooting percentage reached 70%.

Higher NAA concentration (0.6 to 2.0 mg/L) did not show significant differences on number of new root (Fig. 2). In general, the root lengths were higher on media containing IAA (Table 2). In the current study, higher auxin concentration increased rooting percentage as well as root number. This is contrary to the previous findings of Shatnawi et al. (2011b) who reported that lower numbers of *Stevia rebaudiana* new roots were formed at -high IBA concentration. MS medium with 1.5 or 2.0 mg/L IBA produced maximum root formation percentage (75%). Our results demonstrated that the inclusion of IBA, NAA or IAA in the culture medium did not help in shoot multiplication. This result is similar to previous report on *F. carica* (Kumar et al. 1998). Rooting percentage of 55% was recorded on media containing 1.2 to 2.0 mg/L (Table 2). Increased IBA, NAA or IAA concentration increased number of roots per ex-plant increased significantly.

#### **Ex vitro acclimatization:**

Rooted *in vitro* plantlets were successfully acclimatized and transplanted to greenhouse. Successful acclimatized plants did not show any variation in growth or morphological. This method was appeared to produce 80% survival rate when plantlets moved to the greenhouse (Fig. 3).

#### **Effect of pH on microshoot growth:**

The pH of the medium may be a limiting factor for growth. Therefore, pH was evaluated and adjusted between 4 to 6. Among this level, maximum number on newly formed shoots were recorded on the medium adjusted to 5.8. Also maximum shoot length (35.6-39.6) was obtained at pH level 5.8-6.0. Of the six pH tested, 5.8-6.0 was superior to 4.0 to 5.5. pH at 6.0 was found to be the most effective for formation of new shoots. This is because many chemical reactions occur on media at different pH levels. However, change in pH might affect ammonium and nitrate uptake, which could affect nitrate uptake *in vitro* (Dougall, 1980). As reported early by Shibli et al. (1999) and Leifrit et al. (1992), different plant species need different pH for optimal growth.

#### **Conservation of microshoots**

The effect of sucrose in combination with light/dark conditions was examined for the purpose of midterm-conservation of *F. carica* microshoots (Table 4). The experiments presented her showed that *F. carica* could be stored under normal culture conditions. Conservation under light condition gave better results than dark condition, in terms of survival after 20 and 40 weeks, and in terms of regrowth percentage after 40 weeks.

Sucrose was used for conservation of *Amygdalus communis* as reported previously by Shibli et al. (1999), sucrose might reduce metabolic activity pathway.

In the current study increased sucrose concentration in both conditions, decreased the survival after forty-week storage periods. Incorporation of 3% sucrose in the media in combination with light conditions gave the highest survival after 20 weeks (97.6%) and after 40 weeks (95.6%). Also these conditions gave maximum regrowth percentage (89.3%) after 40 weeks. The results showed that *F. carica* can be stored without transfer for up to 40 weeks, without serious losses (Table 4). In this study, no new shoots were obtained during storage periods. This could be due devoid hormone on the medium. Thus the addition of sucrose is advised when cultures are stored for longer periods without subculture.

## Conclusion

Thus the present investigation has resulted in the establishment of a reliable and reproducible protocol of *F. carica* which could be used for mass multiplication as well as for the conservation of fig germplasm. Further study is still required to optimize the protocol and also evaluate genetic stability of stored plant tissues. Additional studies are still needed on cryopreservation and genetic stability of stored cryogenic plant tissues if possible.

Table 1. Influence of different concentration of benzyl amino purine (BAP), kinetin or zeatin on shoot number, and shoot height of *Ficus carica* after six weeks' growth periods.

Growth regulator (mg/L)	Number of new shoot/explant	Shoot height (mm)	Growth regulator (mg/L)	Number of new shoot/explant	Maximum shoot height (mm)
<b>BAP + 0.0 mg/L IBA</b>			<b>BAP + 0.2 mg/L IBA</b>		
<b>0.0</b>	1.1 a	25.9 b	<b>0.0</b>	1.5 b	29.0 b
<b>0.4</b>	3.2 d	20.6 a	<b>0.4</b>	4.2 e	36.6 c
<b>0.8</b>	2.9 c	25.2 b	<b>0.8</b>	3.2 d	36.2 c
<b>1.2</b>	2.6 c	30.5 c	<b>1.2</b>	2.3 c	29.5 b
<b>1.6</b>	2.8 c	30.7 c	<b>1.6</b>	2.8 c	27.7 b
<b>2.0</b>	2.9 c	30.0 c	<b>2.0</b>	1.9 b	27.0 b
<b>kinetin + 0.0 mg/L IBA</b>			<b>kinetin + 0.2 mg/L IBA</b>		
<b>0.0</b>	1.1 a	25.9 b	<b>0.0</b>	1.5 b	29.0 b
<b>0.4</b>	1.1 a	26.1 b	<b>0.4</b>	1.1 a	27.1 b
<b>0.8</b>	1.2 a	26.5 b	<b>0.8</b>	1.2 a	22.5 a
<b>1.2</b>	1.2 a	21.6 a	<b>1.2</b>	1.2 a	22.6 a

<b>1.6</b>	1.1 a	20.6 a	<b>1.6</b>	1.2 a	22.6 a
<b>2.0</b>	1.2 a	20.6 a	<b>2.0</b>	1.2 a	23.9 a
<b>zeatin + 0.0 mg/L IBA</b>			<b>zeatin + 0.2 mg/L IBA</b>		
<b>0.0</b>	1.1a	25.9 b	<b>0.0</b>	1.5 b	29.0 b
<b>0.4</b>	1.3 a	20.5 a	<b>0.4</b>	1.3 a	23.5 a
<b>0.8</b>	1.3 a	20.0 a	<b>0.8</b>	1.2 a	23.0 a
<b>1.2</b>	2.2 b	19.6 a	<b>1.2</b>	1.3 a	12.6 a
<b>1.6</b>	2.1 b	20.8 a	<b>1.6</b>	1.3 a	22.8 a
<b>2.0</b>	2.0 b	24.1 b	<b>2.0</b>	1.2 a	23.1 a

Values represented means; each treatment consisted of 20 replicates, means with the same letter of each column are not significantly different based on least significant difference (LSD) at 0.05 level of probability

Table 2. Influence of different concentration of indole-3- butyric acid (IBA), indole acetic acid (IAA) and naphthalene acetic acid (NAA) on number of shoots, shoot height, number of roots and root length of *in vitro* shoots of *Ficus carica* after six weeks' growth periods on culture.

<b>Growth regulators (mg/L)</b>	<b>Number of new shoot/explant</b>	<b>Shoot height (mm)</b>	<b>Number of root/explant</b>	<b>Root length (mm)</b>	<b>Shoot developing root (%)</b>
<b>IBA</b>					
<b>0.0</b>	1.1	25.9 b	0.0 a	0.0 a	0.0
<b>0.3</b>	1.1	33.6 c	1.0 b	19.5 c	50
<b>0.6</b>	1.2	39.6 d	1.6 b	18.1 c	55
<b>0.9</b>	1.5	42.8 d	1.6 b	18.3 c	55
<b>1.2</b>	1.2	40.9 d	2.3 c	27.6 d	65
<b>1.5</b>	1.2	40.8 d	4.3 e	29.6 d	75
<b>2.0</b>	1.2	43.6 d	4.1 e	10.0 b	75
<b>NAA</b>					
<b>0.3</b>	1.1	28.3 b	1.0 b	7.7 b	35
<b>0.6</b>	1.1	23.9 b	2.1 bc	7.8 b	35

<b>0.9</b>	1.0	28.6 b	3.4 d	9.6 b	45
<b>1.2</b>	1.0	27.2 b	3.4 d	8.8 b	55
<b>1.5</b>	1.0	24.7 b	3.3 c	7.7 b	50
<b>2.0</b>	1.0	25.6 b	3.7 c	8.8 b	55
<b>IAA</b>					
<b>0.3</b>	1.1	28.2 b	1.7 b	5.5 b	65
<b>0.6</b>	1.1	28.7 b	1.8 b	7.2 b	70
<b>0.9</b>	1.2	21.6 a	2.3 c	4.8 b	65
<b>1.2</b>	1.1	22.4 a	3.0 c	6.5 b	70
<b>1.5</b>	1.1	22.0 a	3.6 cd	4.8 a	65
<b>2.0</b>	1.0	23.6 b	3.5 d	4.1 a	60

Values represented means; each treatment consisted of 20 replicates, means with the same letter of each column are not significantly different based on least significant difference (LSD) at 0.05 level of probability.

Table 3. Effect of different pH level on *in vitro* grown *Ficus carica* on number of shoots and shoot length after six weeks on culture.

<b>pH</b>	<b>Number of new shoot/explant</b>	<b>Shoot length (mm)</b>
<b>4.0</b>	1.0 a	15.6 a
<b>4.5</b>	1.0 a	15.9 a
<b>5.0</b>	1.0 a	15.6 a
<b>5.5</b>	3.6 b	33.6 b
<b>5.8</b>	4.1 b	35.6 b
<b>6.0</b>	3.9 b	39.6b

Values represented means; each treatment consisted of 20 replicates, within each column means with the same letter are not significantly different based on least significant difference (LSD) at 0.05 level of probability. Each medium was supplemented with 0.4 mg/l BAP.



Table 4. Influence of different sucrose concentrations on survival and regrowth rate of *in vitro* microshoots of *Ficus carica* pre conserved for 20 or 40 weeks.

Sucrose %	Survival rate after 20 weeks	Survival rate after 40 weeks	Regrowth after after-40 weeks storage
<b>Light condition</b>			
0.0	0.0 a	0.0 a	0.0 a
3.0	97.6 d	95.6 e	89.3 e
6.0	75.3 c	75.0 d	65.6 d
9.0	63.3 c	56.6 c	42.3 c
12	35.6 b	20.0 b	18.6 b
<b>Dark condition</b>			
0.0	0.0 a	0.0 a	0.0 a
3.0	75.6 c	58.7 c	45.6 c
6.0	72.6 c	57.0 c	40.0 c
9.0	70.6 c	55.0 c	39.3 c
12	40.6 b	15.6 b	5.0 a

Values represented means; each treatment consisted of 20 replicates, means with the same letter are not significantly different based on least significant difference (LSD) at 0.05 level of probability.

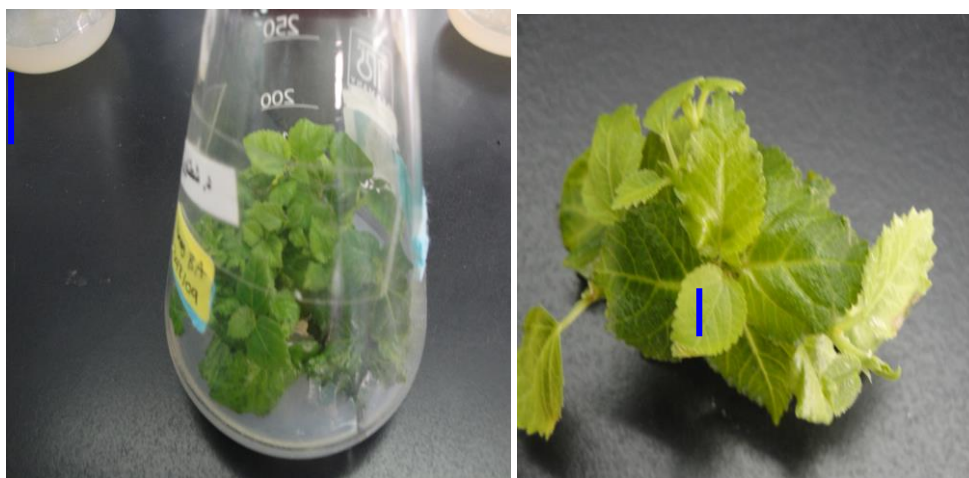


Fig. 1. *In vitro* shoot formation of *Ficus carica*. Multiple microshoot grown on MS containing 0.4 mg/L BAP and 0.2 mg/L IBA after 40-day growth periods. Bar represents 5 mm.





Fig. 2. *In vitro* root formation of *Ficus carica* on MS containing 0.3 mg/L IAA after 40-day growth period. Bar represents 5 mm.

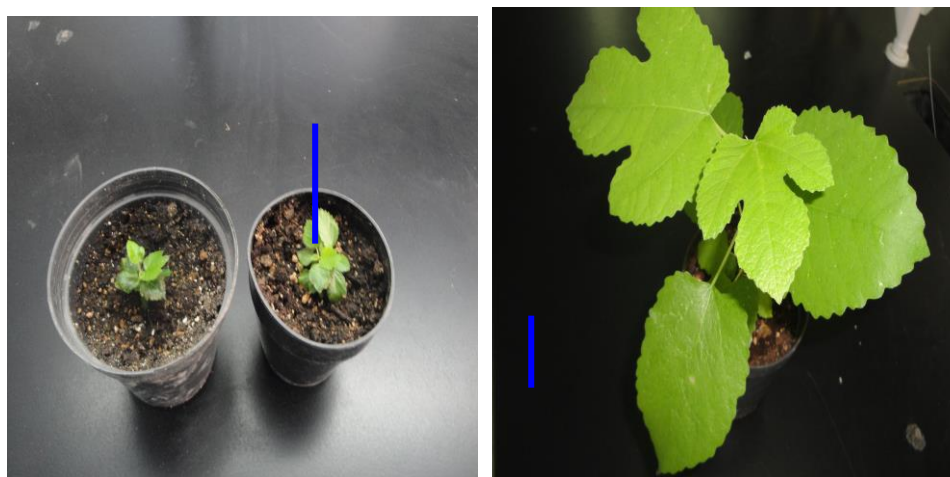


Fig. 3. Acclimatization of *F. carica* plantlets growing in the greenhouse after six weeks. Bar represents 5 cm.

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