*J. Agr. Sci. Tech. (2015) Vol. 17: 1597-1608*

# *In vitro* Propagation of Caprifig and Female Fig Varieties (*Ficus carica* L.) from Shoot-tips

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## ABSTRACT

**Fig trees are threatened by the attack of Fig Mosaic Disease (FMD) on leaves and fruits caused by viruses of several genera. Shoot-tip culture is a convenient method for viral sanitation. For this purpose, a reliable protocol for rapid *in vitro* propagation was developed with shoot-tips of three major Tunisian local fig (*Ficus carica* L.) varieties Zidi (ZDI), Soltani (SNI), Bither Abiadh (BA) and one rare and recalcitrant caprifig Assafri (ASF). For each *in vitro* step, four Murashige and Skoog (MS) media with different combinations of plant regulators were used. The best initiation of shoot-tips with sizes 0.5, 1 and 1.5 mm was obtained on medium $M_3$ containing 0.2 mg $L^{-1}$ Benzyle Amino Purine (BAP), 0.1 mg $L^{-1}$ 1-NaphthaleneAcetic Acid (NAA) and 0.1 mg $L^{-1}$ Gibberellic acid ($GA_3$). The variety (SNI) showed the highest shoot-tip initiation potentialities for the establishment step with 100% of explant development rate. The shoot multiplication and plantlet development were provided by medium $M_6$ with 0.5 mg $L^{-1}$ BAP and 0.1 mg $L^{-1}$ NAA. The highest average of leaf number increase (92 leaves per plant) and proliferation rate (16.91 branches per plant) were reached on $M_6$. The best rooting rate (83.34%) was favored by medium $M_{11}$ with half-strength MS and 1 mg $L^{-1}$ Indole-3-Butyric Acid (IBA). *Ex vitro* rooting of fig plantlets was successfully performed on moist peat with success rate of 90%. The acclimatized fig vitroplants showed high establishment rates (92.1%) and rapid growth on substrates $S_1$ composed by peat without symptoms of virus diseases or morphological abnormalities.**

**Keywords:** Acclimatization, Culture media, Explant size, Micropropagation.

## INTRODUCTION

Fig tree (*Ficus carica* L.) has gained more interests in recent years for its economic importance and medicinal virtues. In Tunisia, fig tree is with remarkable genetic diversity and all cultivars are well adapted to local conditions (Mars *et al*., 2008; 2009). However, several abiotic and biotic constraints limit the development of this fruit crop and contribute to the decrease in revenue and the gradual disappearance of plantations (Saddoud *et al*., 2011). The lack of selected pollinators according to the needs of female cultivars, and the difficulties of their multiplication in fields, are major constraints (Gaaliche *et al*., 2013). Virus diseases, especially fig mosaic disease, are actually a threat and spread in all areas of production (Saddoud *et al*., 2007); which inhibits the development of healthy orchards and limits the production (Ashihara *et al*., 2004). Improvement of farming techniques (Fraguas *et al*., 2004), the sanitation of local varieties (Mars *et al*., 2008), and the large-scale production of good quality and healthy fig plants and pollinators by meristem or shoot tip culture (Pontikis and Melas, 1986; Gella *et al*,. 1998; Günver and Ertan, 1998)

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[ DOR: 20.1001.1.16807073.2015.17.6.6.7 ]

seem to be basic requirements for successful commercial orchards (Pasqual and Ferreira, 2007). This requires the mastery of rapid micropropagation of local figs. Tissue culture is a basic method for the propagation of plant material with high multiplication rates. In Tunisia, *in vitro* protocols for rapid regeneration of virus-free fig trees were not developed. Therefore, the main objective of this study was the establishment and optimization of a reliable protocol for *in vitro* regeneration of virus-free fig plants from three important local female varieties and one selected caprifig (pollinator).

## MATERIALS AND METHODS

Four to five cm length shoots were taken from fig mosaic symptomatic female adult trees of three local varieties Zidi (ZDI), Soltani (SNI), Bither Abiadh (BA) and the pollinator Assafri (ASF) (Bayoudh *et al*., 2012). All samples were washed under running tap water for 15 minutes. Then, the samples were kept for 25 minutes in 10% NaOCl solution with 2 drops of Tween-20, followed by three rinses of 10 minutes in sterile distilled water. Shoot-tips of 0.5, 1.0 and 1.5 mm were excised from the aseptic explants (Figure 1) and were immediately placed on 4 Murashige and Skoog (MS) (1962) basal salts supplemented with 30 g $L^{-1}$ sucrose, 7 g $L^{-1}$ Agar, 90 mg $L^{-1}$ Phloroglucinol (PG) (Bio Basic Canada Inc., Ontario) and different growth regulators for the study of their initiation (Figure 2).

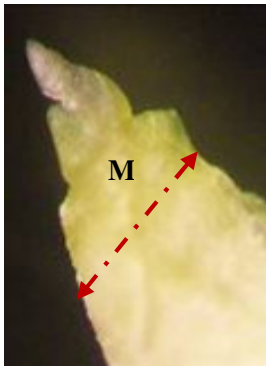


**Figure 1.** Shoot-tip of fig (M) (G: 32x).

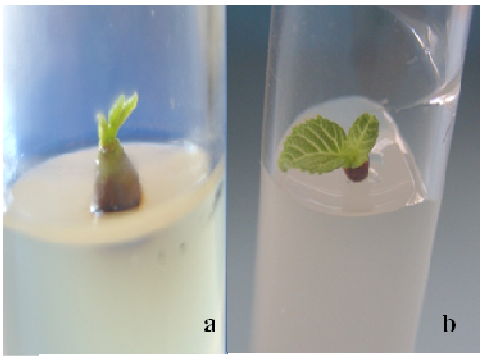


**Figure 2.** Initiation of fig shoot-tip on medium $M_3$ (a, b).

The initiation media were:

$M_1$: MS+0.2 mg $L^{-1}$ BAP+0.1 mg $L^{-1}$ NAA+0.1 mg $L^{-1}$ Kin+PG; $M_2$: MS+0.2 mg $L^{-1}$ BAP+0.1 mg $L^{-1}$ NAA+0.1 mg $L^{-1}$ IsoPentyl Adenosine (IPA)+PG ; $M_3$: MS+0.2 mg $L^{-1}$ BAP+0.1 mg L-$^{1}$ NAA+0.1 mg $L^{-1}$ $GA_3$+PG, and $M_4$: MS+0.2 mg $L^{-1}$ BAP+0.1 mg $L^{-1}$ 2,4-dichlorophenoxy acetic acid (2,4-D)+PG.

For the proliferation and the elongation of the *in vitro* shoots, the following media were tested:

$M_5$: MS+0.5 mg $L^{-1}$ BAP+0.1 mg $L^{-1}$ IBA+0.1 mg $L^{-1}$ $GA_3$; $M_6$: MS+0.5 mg $L^{-1}$ BAP+0.1 mg $L^{-1}$ NAA+0.1 mg $L^{-1}$ $GA_3$; $M_7$: MS+0.5 mg $L^{-1}$ Kin+0.1 mg $L^{-1}$ IBA+0.1 mg $L^{-1}$ $GA_3$, and $M_8$: MS+0.5 mg $L^{-1}$ Kin+0.1 mg $L^{-1}$ NAA+0.1 mg $L^{-1}$ $GA_3$.

Rooting of plantlets was studied on:

$M_9$: MS+1 mg $L^{-1}$ IBA; $M_{10}$: MS+1 mg $L^{-1}$ NAA; $M_{11}$: $MS^{1/2}$ (Half strenth of MS)+1 mg $L^{-1}$ IBA, and $M_{12}$: $MS^{1/2}$+1 mg $L^{-1}$ NAA.

The pH of all media was adjusted to 6.0 prior to autoclaving for 20 minutes at 121°C and 1.1 kg $cm^{-2}$. Cultures were maintained in a growth chamber at 25±1°C under 16 hours photoperiod with a light intensity of 40 µmol $m^{-2}$ $s^{-1}$ provided by cool white fluorescent lamps.

### Recorded Parameters for Proliferated and Acclimatized Plants

The number of shoots per each explant was counted at the multiplication stage.





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Well- developed fig plantlets of female varieties SNI and ZDI and the pollinator ASF were potted on moist peat and covered by transparent plastic film for *ex vitro* rooting. For the acclimatization step, well-rooted vitroplants were washed and placed on two substrates: ($S_1$) Composed by only peat, and ($S_2$) By a 2:1 mixture of peat and perlite. Survival rates (after four and ten weeks) were recorded for all studied varieties. Plant growth during acclimatization was studied for Smyrna type varieties ZDI and SNI. On each substrate, we determined the average increases in height, leaf number, and proliferation rate of acclimatized plants after 6 weeks. The survived plants were transferred to larger pots under greenhouse covered by insect proof sheet.

### Data Analysis

All experiments were carried out in a factorial statistical scheme and a completely randomized design. Each treatment was replicated three times. Data were submitted to analysis of variance (ANOVA), and means were compared by Duncan test, using software SPSS (SPSS Inc., Ver.11.0).

## RESULTS AND DISCUSSION

### Effect of Explant Size and Media Composition

Shoot-tip size is a very important factor in the elimination of viruses from plants (Verbeek *et al*., 1995; Panattoni *et al*., 2013). In our attempts of fig sanitation via shoot-tip culture, we studied the effect of three shoot-tip sizes (0.5, 1, and 1.5 mm) (Bayoudh *et al.*, 2013) on their survival and development on medium $M_3$. For all studied varieties, shoot-tip size appeared to significantly affect their evolution rates (Table 2). For the pollinator ASF, the highest rate of recovery (96%) was obtained with shoot-tip size 0.5 mm. For the other varieties, this rate was obtained with larger sizes: 1 mm for ZDI and BA (79 and 73.33%, respectively) and 1.5 mm for SNI (95.22%). It seems that the size of excised shoot-tip is not only important to produce virus-free plants of many crops, but also to determine the survivability of the explants in culture (Malaurie *et al*., 1995; Manganaris *et al*., 2003). In this context, Parmar *et al*. (2013) mentioned that *in vitro* shoot growth of *Clerodendrum inerme* was higher with larger size explants than the smaller one. This may be due to the high number of leaf primordia in large shoot-tips. Sahraroo *et al*. (2009) encountered many difficulties to regenerate *Ficus carica* plantlets from 0.2 to 0.4 mm meristems since they failed to grow on the media. For all varieties, abundant necroses were observed at the bases of large explant sizes and the highest necrosis rate (76.45%) was recorded at the base of 1.5 mm shoot-tips of the male variety ASF.

### Establishment Media

Effect of establishment media was studied on small size shoot-tips (0.5 mm) of varieties ZDI, BA, SNI and pollinator ASF. These explants were cultured and initiated on the establishment media $M_1$, $M_2$, $M_3$ and $M_4$. During the first 2 weeks of establishment, explants browning were noticed. Its negative effects on the explants were reduced by PG, which acts as antioxidant and increases growth rate of the cultures (Hepaksoy and Aksoy, 2005). Similar behavior of small fig shoot tip explants on establishment media was observed by Gella *et al*. (1998) and Dhage *et al*. (2012), who reported that explant browning was avoided by the use of the antioxidants. After eight weeks of culture, growth and emergence of shoot apices were highly dependent to the composition of establishment medium. Significant interaction was noted between the 4 varieties and the establishment media concerning shoot-tip necrosis rate. The variety Soltani (SNI) showed better growth and less necrosis and calli at the bases of explants than the other three varieties (Table 1). For SNI, the highest rate of shoot-tip survival and





[ DOR: 20.1001.1.16807073.2015.17.6.6.7 ]

**Table 1.** Effect of shoot-tip size of different varieties of fig on necrosis, callus, and development rates.

| Variety | Size (mm) | % Necrosis | % Callus at explant bases | % Development |
|---|---|---|---|---|
| ZDI [a] | 0.5 | 46.67 ± 21.66 | 23.89 ± 3.89 | 61.11 ± 11.11 |
| | 1 | 36.82 ± 4.55 | 33.39 ± 13.34 | 79.00 ± 11.59 |
| | 1.5 | 64.31 ± 12.87 | 55.10 ± 17.5 | 70.49 ± 3.32 |
| BA [b] | 0.5 | 58.50 ± 11.64 | 31.10 ± 1.1 | 67.77 ± 19.28 |
| | 1 | 43.33 ± 16.66 | 33.33 ± 8.81 | 73.33 ± 8.81 |
| | 1.5 | 70.00 ± 5.77 | 60.00 ± 5.77 | 56.67 ± 12.01 |
| SNI [c] | 0.5 | 5.71 ± 3,68 | 4.28 ± 2,97 | 90.00 ± 6,54 |
| | 1 | 31.42 ± 5.53 | 7.14 ± 5.65 | 55.71 ± 2.97 |
| | 1.5 | 6.18 ± 3.03 | 4.28 ± 2.97 | 95.22 ± 3.07 |
| ASF [d] | 0.5 | 72.67 ± 8.19 | 37.67 ± 16.89 | 96.00 ± 4.0 |
| | 1 | 69.79 ± 4.01 | 48.36 ± 20.54 | 92.63 ± 3.68 |
| | 1.5 | 76.45 ± 10.50 | 46.65 ± 10.18 | 87.96 ± 7.23 |
| Statistical significance: | | | | |
| Size effect | | NS | NS | * |
| Interaction Variety×Size | | ** | ** | ** |

[a] Zidi; [b] Bither Abiadh; [c] Soltani, [d] Assafri. *: Significant differences (Duncan, P≤ 0.05), **: Highly significant differences (Duncan, P≤ 0.01), NS: Not significant differences. The values are compared vertically.

**Table 2.** Effect of four establishment media on shoot-tip explants development of different varieties of fig**.**

| Variety [a] | Medium | % Necrosis | % Callus at explant bases | % Development |
|---|---|---|---|---|
| | $M_1$ | 53.33 ± 3.33 | 39.17 ± 0.83 | 59.17 ± 12.27 |
| ZDI [a] | $M_2$ | 53.33 ± 12.01 | 77.59 ± 7.21 | 16.67 ± 3.33 |
| | $M_3$ | 46.67 ± 21.66 | 23.89 ± 3.89 | 61.11 ± 11.11 |
| | $M_4$ | 75.17 ± 13.10 | 62.20 ± 23.19 | 31.83 ± 16.53 |
| | $M_1$ | 78.33 ± 11.66 | 38.33 ± 7.26 | 41.67 ± 22.04 |
| BA [b] | $M_2$ | 66.67 ± 17.63 | 58.33 ± 13.64 | 73.33 ± 14.52 |
| | $M_3$ | 58.50 ± 11.64 | 31.10 ± 1.1 | 67.77 ± 19.28 |
| | $M_4$ | 53.33 ± 18.55 | 40.00 ± 0 | 66.67 ± 18.55 |
| | $M_1$ | 5.00 ± 2,67 | 8.75 ± 5,80 | 100 ± 0 |
| SNI [c] | $M_2$ | 0 ± 0 | 0 ± 0 | 100 ± 0 |
| | $M_3$ | 5.71 ± 3,68 | 4.28 ± 2,97 | 90 ± 6,54 |
| | $M_4$ | 2.22 ± 2,22 | 0 ± 0 | 96.66 ± 3,33 |
| | $M_1$ | 40.00 ± 11.54 | 40.00 ± 20.0 | 86.67 ± 13.33 |
| ASF [d] | $M_2$ | 65.77 ± 22.9 | 81.30 ± 13.53 | 66.30 ± 16.15 |
| | $M_3$ | 72.67 ± 8.19 | 37.67 ± 16.89 | 96.00 ± 4.0 |
| | $M_4$ | 61.67 ± 3.34 | 20.37 ± 15.15 | 83.77 ± 11.11 |
| Statistical significance: | | | | |
| Media effect | | ** | NS | NS |
| Interaction Varieties×Media | | ** | NS | * |

[a] Zidi; [b] Bither Abiadh; [c] Soltani, [d] Assafri. * Significant differences (Duncan, P≤ 0.05), ** Highly significant differences (Duncan, P≤ 0.01), NS: Not significant differences. The values are compared vertically.





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evolution (100%) was achieved on the media $M_1$ and $M_2$. However, on $M_2$, no necrosis or callus formation at the bases of shoot-tips occurred. Zidi (ZDI) showed low evolution rates on all initiation media and the lowest rate (16.67%) was obtained with $M_2$. The majority of explants were susceptible to necrosis on all culture media. The highest rate of necrosis (78.33%) was recorded with (BA) on $M_1$. Assafri (ASF) explants gave the highest rate of callus at their bases (81.3%) on $M_2$. Among the four tested establishment media, $M_2$ and $M_3$ were the most adequate for shoot-tip development of ZDI, BA, SNI and ASF, but $M_3$ supplemented with $GA_3$ was the best one sine it produced lower rates of callus at the explant bases. Similar results were reported by Fraguas *et al.* (2004).

## Proliferation Media

Plantlets of the four tested varieties ZDI, BA, SNI and ASF, obtained during the initiation phase, were transferred to the multiplication media $M_5$, $M_6$, $M_7$, and $M_8$ containing BAP and Kinetin. Statistical analysis of shoot development and growth on all proliferation media for 10 weeks showed that shoot proliferation was significantly dependent on varieties and culture media (Table 3).

Similar results were obtained by Hepaksoy and Aksoy (2006) and Mustafa and Taha (2012). The variety ZDI presented the highest proliferation potentialities: average number of branches per plant (16.91), average number of leaves per plant (92) and average increase in plant height (16.33 mm) (Table 3). The highest rates of shoot proliferation for varieties BA (7.08 branches per plant), ZDI (16.91 branches per plant) and pollinator ASF (6.83 branches per plant) were obtained with the medium $M_6$ containing BAP, IBA, and $GA_3$. On this medium, the shoots were well developed,

**Table 3.** Effect of four proliferation media on *in vitro* plant growth of four genotypes of fig.

| Variety | Medium | Average increase of plant height (mm) | Number of leaves | Number of newly formed shoots |
|---|---|---|---|---|
| SNI [a] | $M_5$ | 9.01 ± 1.37 a | 48.41 ± 9.21 a | 9.00 ± 1.17 a |
| | $M_6$ | 9.60 ± 1.75 ab | 30.58 ± 4.84 a | 7.17 ± 1.36 a |
| | $M_7$ | 5.88 ± 1.27 b | 5.91 ± 3.15 b | 1.08 ± 0.31 b |
| | $M_8$ | 6.93 ± 2.28 ab | 10.33 ± 6.45 c | 1.67 ± 1.02 c |
| BA [b] | $M_5$ | 9.79 ± 0.82 a | 34.31 ± 5.18 a | 6.83 ± 0.90 a |
| | $M_6$ | 7.83 ± 1.03 ab | 26.77 ± 5.80 a | 7.08 ± 1.16 a |
| | $M_7$ | 9.59 ± 1.00 b | 8.70 ± 4.79 b | 3.67 ± 1.02 b |
| | $M_8$ | 5.24 ± 1.71 ab | 7.36 ± 2.60 c | 1.42 ± 0.33 c |
| ZDI [c] | $M_5$ | 17.0 ± 0.82 a | 66.91 ± 7.25 a | 13.41 ± 1.49 a |
| | $M_6$ | 11.92 ± 0.97 ab | 92.00 ± 8.83 a | 16.91 ± 1.98 a |
| | $M_7$ | 10.54 ± 2.05 b | 61.17 ± 13.35 b | 9.91 ± 1.82 b |
| | $M_8$ | 16.33 ± 2.4 ab | 26.67 ± 4.30 c | 1.91 ± 0.39 c |
| ASF [d] | $M_5$ | 6.94 ± 0.99 a | 28.58 ± 4.14 a | 6.75 ± 0.85 a |
| | $M_6$ | 7.60 ± 1.38 ab | 28.41 ± 8.31 a | 6.83 ± 1.43 a |
| | $M_7$ | 4.74 ± 1.35 b | 7.75 ± 2.13 b | 1.33 ± 0.65 b |
| | $M_8$ | 3.74 ± 1.87 ab | 6.41 ± 3.82 c | 1.50 ± 0.85 c |
| Statistical significance: | | | | |
| Variety effect | | ** | ** | ** |
| Media effect | | * | ** | ** |
| Interaction Varieties×Media | | * | ** | ** |

[a] Soltani, [b] Bither Abiadh; [c] Zidi; [d] Assafri. *: Significant differences (Duncan, P≤ 0.05). **: Highly significant differences (Duncan, P≤ 0.01), The values are compared vertically. Means with different letter in a row are statistically different (Duncan, P≤ 0.05).





[ DOR: 20.1001.1.16807073.2015.17.6.6.7 ]

greenish and with small amount of basal calli (Figure 3). BAP is a very critical cytokinin for proliferation. It was reported to stimulate multiple shoot formation in vitroplants (Saidi *et al*., 2007; Singh *et al*., 2010; Karimpour *et al*., 2013; Zuraida *et al*., 2014). Also, the proliferation medium $M_6$ contained $GA_3$, which is considered to be essential not only to improve the growth of the explants, but to increase significantly the number of nodes and leaves (Rostami and Shahsava, 2012). For SNI, the best proliferation rate (9.0 branches per plant) was provided by the medium $M_5$. The lowest rates were, generally, obtained by the medium $M_8$ containing Kinetin as reported by Mustafa and Taha (2012). On medium $M_8$, the shoots were stunted, with callus and some yellowish leaves. This may be due to reaction effects of Kinetin and NAA on plant respiratory metabolism (Akemine *et al*., 1975). The best plantlets elongation rate and increase in number of leaves were mainly provided by media $M_5$ and $M_6$ and the lowest values were recorded on $M_7$ and $M_8$. It appeared that BAP ($M_5$ and $M_6$) had significant higher effects on the vitroplants. Similar results have been reported by Kumar *et al*. (1998) and Nobre and Romano (1998) for the regeneration of fig vitroplants from apical buds on media containing BAP.

**Figure 3.** Proliferation and elongation of fig shoots on medium $M_6$.

## Rooting Media

Single vigorous plantlets of each variety were transferred to different rooting media $M_9$, $M_{10}$, $M_{11}$ and $M_{12}$ to induce and develop the root system (Figure 4). Results showed that plant rooting rate was significantly impacted by the variety and rooting media effects (Table 4). The highest percentages of rooted plants (83.34%) occurred for SNI on $M_{10}$ and ASF on $M_{11}$. In addition, $M_{11}$ contained half strength of MS and 1 mg $L^{-1}$ IBA gave best results for ZDI plants (75%). These results were in conformity with previous reports (Kumar *et al*., 1998; Yancheva *et al*., 2005; Soliman *et al*., 2010) where *in vitro* rooting of fig vitroplants was facilitated by 1 to 2 mg $L^{-1}$ IBA containing media. $M_{11}$ induced development of longest roots in ASF (52.4 mm) and variety SNI (39.2 mm) (Table 5). Furthermore, highest number of secondary roots of ZDI (11.5), ASF (9.7) and SNI (10.7) plantlets were recorded on $M_{11}$ medium. Therefore, the composition of this medium (with reduced nitrogen and auxins) was, globally, the most suitable for fig vitroplant rooting. Similar findings for other plant species were mentioned by many authors (Ebrahim *et al*.,

**Figure 4.** Multiple and long roots of fig vitroplant induced on medium $M_{11}$.





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**Table 4.** Fig plantlet root development.

| Variety | Medium | Rooting rate (%) | Root length (mm) | Principal root number/ plant | Secondary root number/plant |
|---|---|---|---|---|---|
| ZDI [a] | M9 | 50.00 ± 15.07 b | 26.8 ± 4.25 b | 2.50 ± 0.67 b | 2.50 ± 1.62 b |
| | M10 | 33.34 ± 14.21 b | 35.5 ± 12.75 ab | 4.00 ± 1.73 a | 11.00 ± 5.80 a |
| | M11 | 75.00 ± 13.05 a | 28.20 ± 5.61 a | 2.55 ± 0.61 ab | 11.50 ± 3.56 a |
| | M12 | 8.34 ± 8.34 b | 18.60 ± 0.0 ab | 1.00 ± 0 a | 0 ± 0 b |
| BA [b] | M9 | 8.34 ± 8.34 b | 1.60 ± 0 b | 1.00 ± 0 b | 0 ± 0 b |
| | M10 | 16.67 ± 11.23 b | 9.90 ± 0.29 ab | 3.50 ± 1.45 a | 7.00 ± 1.73 a |
| | M11 | 58.34 ± 14.86 a | 22.50 ± 5.49 a | 2.30 ± 0.37 ab | 5.70 ± 1.64 a |
| | M12 | 75.00 ± 13.05 b | 22.50 ± 3.33 ab | 3.80 ± 0.48 a | 6.00 ± 1.39 b |
| ASF [c] | M9 | 41.66 ± 14.86 b | 18.80 ± 2.80 b | 1.60 ± 0.26 b | 3.20 ± 1.22 b |
| | M10 | 50.00 ± 15.07 b | 34.50 ± 5.18 ab | 3.57 ± 0.57 a | 8.57 ± 2.80 a |
| | M11 | 83.34 ± 11.23 a | 52.40 ± 6.32 a | 3.40 ± 0.43 ab | 9.70 ± 2.16 a |
| | M12 | 41.67 ± 14.86 b | 37.10 ± 8.88 ab | 3.37 ± 1.19 a | 7.00 ± 2.73 b |
| SNI [c] | M9 | 58.34 ± 14.86 a | 35.10 ± 5.91 b | 2.50 ± 0.37 b | 5.00 ± 1.0 b |
| | M10 | 83.34 ± 11.23 b | 22.20 ± 3.68 ab | 2.90 ± 0.68 a | 10.40 ± 4.04 a |
| | M11 | 75.00 ± 13.05 a | 39.23 ± 6.30 a | 3.40 ± 0.78 ab | 10.70 ± 1.64 a |
| | M12 | 50.00 ± 15.07 b | 37.80 ± 4.03 ab | 3.50 ± 1.05 a | 2.00 ± 0.57 b |
| Statistical Significance: | | | | | |
| Media effect | | ** | * | * | * |
| Variety×media | | NS | NS | NS | NS |

[a] Zidi; [b] Bither Abiadh; [c] Assafri, [d] Soltani. *: Significant differences (Duncan, P≤ 0.05), NS: Not significant differences. The values are compared vertically. Means with different letter in a row are statistically different (Duncan, P≤ 0.05).

**Table 5.** Development of *ex vitro* rooted fig plants on peat after six weeks.[a]

| Variety | Mean plant height (mm) | Leaf number/Plant |
|---|---|---|
| ZDI [a] | 126.10 ± 12.20 | 2.10 ± 0.69 |
| SNI [b] | 91.07 ± 12.79 | 1.91± 0.73 |
| ASF [c] | 104.59 ± 24.01 | 1.45± 0.54 |
| Differences among varieties | NS | NS |

[a] Zidi, [b] Soltani, [c] Assafri. The values are compared vertically, NS: Not significant differences.

2007; Othmani *et al*., 2010 and Toppo *et al*., 2012). For (BA), the best rooting rate (75%) was obtained with $M_{12}$. Furthermore, on $M_{12}$, we obtained strong root system. The plants continued to grow well and to proliferate on this medium and, consequently, they were greenish and with an average of about 6 brunches per plant. Globally, a significant effect of rooting media for root length and number of principal and secondary roots of vitroplants was recorded. The highest average number of main roots (4) was obtained on $M_{10}$ (Table 4).

## Acclimatization Step

### *Ex vitro* Rooting

After six weeks, we noticed that for all varieties, 90% of plants were successfully rooted. Throughout *ex vitro* rooting period, plantlets continued to grow properly. Strategy adopted in the present work was in agreement with *ex vitro* rooting of Blueberry (Guang-jie *et al*., 2008). Bhatia *et al*. (2002) reported that best results for *ex vitro* Stackhousia plantlets reflects the potential





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effects of combining *ex vitro* and hardening in one step, with view to reducing costs of vitroplants. The highest averages of increase in plant height (126.1 mm) and leaf number (2.1) were recorded for ZDI (Table 5). The rooted plants were transferred to larger pots and maintained under the insect proof greenhouse.

### Effects of Substrates on Survival Rate

For all varieties, on the two substrates $S_1$ and $S_2$, the survival rates recorded following the first four weeks ranged from 81.39 to 100%, while the survival rates recorded in the tenth week of transplantation extended from 63.15 to 92.1% (Table 6). This may be due essentially to the gradual loss of some acclimatized plants due to the relatively hard environment as compared to the *in vitro* conditions (Hajong *et al*., 2013). As reported by many authors (Demiralay *et al*., 1998; Kumar *et al.*, 1998; Hepaksoy and Aksoy, 2005; Fraguas *et al.*, 2012), the final survival rates of rooted acclimatized plants vary according to the varieties and the substrates. The highest final survival rate (92.1%) and the lowest final survival rate (63.15%) were recorded for ZDI, respectively, on $S_1$ and $S_2$ (Table 6). Generally, $S_1$, which was composed of only peat, allowed the best survival rates. This substrate contained appropriate amount of nutrients (Fraguas *et al*., 2012); and had good water retention capacity, which played an important role in plant growth (Mengesha *et al*., 2013).

### Effect of Substrates on Plant Growth

Plants of both varieties ZDI and SNI presented better growth on $S_1$. The highest increase in plant height (15.48 mm) and branching number (0.7) were recorded with $S_1$, whereas the highest leaf number (1.9) was obtained with $S_2$ (Table 7). These results of acclimatized plant growth are considered satisfactory if compared to those obtained by

**Table 6.** Survival rates (%) of acclimatized fig plants on substrates $S_1$ and $S_2$.

| | ZDI[a] | | SNI[b] | | BA[c] | | ASF[d] | |
|---|---|---|---|---|---|---|---|---|
| | Survival rate during first 4 weeks | Final survival rate | Survival rate during first 4 weeks | Final survival rate | Survival rate during first 4 weeks | Final survival rate | Survival rate during first 4 weeks | Final survival rate |
| $S_1$: Peat | 100 ± 0 | 92.10 ± 4.43 | 96.05 ± 2.24 | 67.10 ± 5.42 | 96.07 ± 2.74 | 86.27 ± 4.86 | 96.49 ± 1.73 | 85.96 ± 4.52 |
| $S_2$: Peat+ Perlite | 100 ± 0 | 63.15 ± 8.03 | 81.39 ± 4.51 | 66.17 ± 5.78 | 94.73 ± 5.71 | 89.47 ± 7.23 | 87.14 ± 3.15 | 64.28 ± 3.26 |
| Differences between substrates | NS | ** | NS | NS | NS | NS | NS | ** |

[a] Zidi; [b] Soltani, [c] Bither Abiadh; [d] Assafri. **: Highly significant differences ($P \leq 0.01$), NS: Not significant differences. The values are compared vertically.





[ DOR: 20.1001.1.16807073.2015.17.6.6.7 ]

**Table 7.** Growth of acclimatized plants on $S_1$ and $S_2$.

| | ZDI [a] | | | SNI [b] | | |
|---|---|---|---|---|---|---|
| | Plant height (mm) | Leaf number | Branching number | Plant height (mm) | Leaf number | Branching number |
| $S_1$: Peat | 15.48 ± 2,42 | 1.54 ± 0.43 | 0.45 ± 0.15 | 11.36 ± 2.05 | 1.5 ± 0.8 | 0.7 ± 0.3 |
| $S_2$: Peat+Perlite | 13.89 ± 2.02 | 1.0 ± 0.38 | 0.18 ± 0.18 | 9.67 ± 2.16 | 1.9 ± 1.1 | 0.64 ± 0.27 |

[a] Zidi, [b] Soltani

Fraguas *et al*. (2012) who obtained 8.92 mm as the best plant height of plantlets acclimatized during 60 days on 5 different substrates.

Acclimatized plants transferred to the insect proof greenhouse showed good growth. They formed thick trunk, multiple branching and new leaves. No variations in growth or morphological characteristics were detected.

## CONCLUSIONS

A suitable protocol for rapid multiplication of important local fig varieties of Smyrna type (Zidi and Soltani), San Pedro type (Bither Abiadh) and the rare and recalcitrant pollinator Assafri from shoot-tips was developed and optimized. Since this protocol is to be applied for sanitation of local fig varieties, the small shoot-tip size of 0.5 mm height was the most preferred size. The proliferation of fig shootlets was mainly provided by medium $M_6$ containing small amounts of BAP and NAA. With this optimized protocol, e*x vitro* rooting of vitroplants on humidified peat was successfully feasible and allowed high rooting rates, reached 90% with healthy roots, which resulted in reduction of plant micro-propagation period. The micro-propagated plants were FMD-symptoms free and had normal morphological aspects. No abnormalities were noticed. Nevertheless, molecular analysis to test their genetic stability is needed. Following these tests, this optimized protocol for large-scale production of good quality and healthy female fig and pollinator plants will be applied for successful establishment of commercial production.

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[ DOR: 20.1001.1.16807073.2015.17.6.6.7 ]

# تکثیر درون شیشه ای بَرآنجیر و ارقام انجیر ماده (*Ficus carica* L.) از نوک شاخه ها

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## چکیده

برگ ها و میوه های درختان انجیر در تهدید حمله مرض موزائیک انجیر(FMD) قرار دارند که توسط ویروس هایی از چند جنس ایجاد می شود. کشت نوک شاخه (یا ساقه) روش راحتی برای بهسازی و عاری کردن از ویروس است. به این منظور، دستورالعملی قابل اعتماد برای تکثیر سریع درون شیشه ای نوک شاخه های سه رقم محلی و اصلی انجیر تونس (Ficus carica L.) به نام های زیدی (ZDI)، سلطانی (SNI) و بیذر ابیاد (BA) و یک بَرآنجیر گرده افشان مقاوم و کمیاب به نام عصافری (ASF) تهیه شد. در هر گام از این فرایند درون شیشه ای، چهار بستر رشد Murashige and Skoog (MS) با ترکیب های مختلف تنظیم کننده های رشد گیاه به کار گرفته شد. بهترین فرایند آغازین نوک شاخه ها با اندازه های ۰/۵، ۱ ، و ۱/۵ میلی متر روی بستر $M_3$ به دست آمد که حاوی ۰/۲ میلی گرم در لیتر بنزیل آمینو پورین (BAP)، ۰/۱ میلی گرم در لیتر نفتالین استیک اسید (NAA) و ۰/۱ میلی گرم در لیتر جیبرالیک اسید ($GA_3$) بود. رقم SNI با نرخ رشد ۱۰۰٪ ریز نمونه بیشترین فرایند آغازین نوک شاخه ها را برای مرحله استقرار نشان داد. تکثیر ساقه و رشد گیاهچه در بستر $M_6$ بود که حاوی ۰/۵ میلی گرم در لیتر BAP و ۰/۱ میلی گرم در لیتر NAA بود. بالاترین میانگین افزایش تعداد برگ (۹۲ برگ در هر گیاه) و شاخه زنی( ۱۶/۹۱ شاخه در هر گیاه) روی بستر $M_6$ رخ داد. بهترین نرخ ریشه زنی (۸۳/۳۴٪) در بستر $M_{11}$ ثبت شد که حاوی نیم-قدرت MS و ۱ میلی گرم در لیتر ایندول-۳-بوتیریک اسید (IBA) بود. ریشه زایی گیاهچه های انجیر در شرایط طبیعی( مزرعه ای) روی پیت مرطوب با موفقیت ۹۰٪ انجام شد. گیاهچه های سازگارشده انجیر های درون شیشه ای نرخ استقرار زیاد (۹۲/۱٪) و رشدی سریع روی بستر S1 که حاوی پیت بود نشان دادند و علایم امراض ویروسی یا نابهنجاری های شکلی در آنها مشاهده نمی شد.





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